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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patent Examining Operations

Applicant: Johnson, S.

Serial No: 08/290,592 Art Unit: 1806

Filed: August 15, 1994 Examiner: Krikorian, J.

Title: Human-Murine Chimeric Antibodies Against Respiratory Syncitial Virus

Assistant Commissioner for Patents
Washington, D.C. 20231

Declaration of Leslie S. Johnson Under 37 CFR 1.131

Dear Sir:

1. I, Leslie S. Johnson Ph.D., citizen of the United States of America, and residing at 13545 Ambassador Drive, Gewrmantown, PA 20874; state and declare that upon infromation and belief that I am the inventor of the invention described and claimed in U.S. Serial No. 08/290,592, entitled "Human-Murine Chimeric Antibodies Against Respiratory Syncitial Virus".

2. This Declaration is submitted to establish conception of the invetion in this application prior to March 1991, which is the effective date of Tempest *et al.*, cited by the Examiner and to establish reasonable diligence from a date prior to the effective date until Applicant's actual reduction to practice.

3. Attached hereto is Exhibit 1 which establishes a fully formed conception of the invention. It is an SBIR grant application(assignment number 1R43AI30300-01A1) which was submitted and receipt by the National Institutes of Health prior to March 1991. See particularly the bottom half of page 10 and the Experimental Design and Methods section at pages 14-18.

4. Attached hereto are Exhibits 2-4 which establish diligence in the completion of the invention from a time prior to the effective date of Tempest *et al.* continuously up to the date of the actual reduction to practice of the invention, by production of the humanized-mouse chimeric antibody. These documents are as follows:

Exhibit 2:

These notebook pages document work performed by Ms. Lisa Bennett on the cloning and manipulation of the murine anti-RSV MAb 1129 in preparation for humanization. Specifically detailed are the PCR amplification of the 1129 heavy and light variable regions, the insertion of these PCR fragments into plasmid vectors and the DNA sequencing of the resultant inserts. This work was particularly important because she confirmed the existence of additional variable region sequences from the parental myeloma cell line used to make the 1129 hybridoma. These pages document the construction of a single-chain Fv form of the molecule. The variable regions were then combined with the appropriate human constant region gene segment for eventual comparison to hu1129. Also the pages document the construction of a vector for the in-frame insertion of synthetic humanized V-region gene segments.

Exhibit 3:

These notebook pages documents work done by Mr. David Pfarr on the construction and evaluation of a eukaryotic expression vector capable of the expression of humanized anti-RSV MAb's. The vector needed to be capable of both transient and stable expression and to facilitate expression of heavy and light chain genes either by co-transfection or from the same plasmid; both under the control of a strong enhance/promoter.

Exhibit 4:

These pages document the expression and assay of humanized anti-RSV MAb hu1308F. Particularly they document the ability of hu1308F to bind to and thus immuno-precipitate radioactively labeled RSV F-protein expressed from a vaccinia virus construct. In addition they detail the transient expression of hu1129 and the generation of stable cell lines expressing hu1308F and hu1129.

5. This Declaration is submitted prior to final rejection.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereupon.

5/16/96
Date

Leslie S. Johnson, Ph.D.

FIRST CLASS CERTIFICATE

I hereby certify that this correspondence is being deposited today with the U.S. Postal Service as First Class Mail in an envelope addressed to:

Assistant Commissioner for Patents
Washington, DC 20231

Charles J. Herron 5/21/96
Charles J. Herron, Esq. **Date**

Respectfully submitted,

Charles J. Herron

Charles J. Herron, Esq.
Reg. No. 28,019

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Six Becker Farm Road
Roseland, NJ 07068

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Exhibit 1

FROM

IRG: SSS 4
SPECIAL SS 4
DR. NABEEH MOUHAD, EXEC. SECY.
DRG/NATIONAL INSTITUTES OF HEALTH
RM 2A04 5333 WESTBARD AVENUE
BETHESDA, MD 20892 (301) 496-7310

A GRANT APPLICATION HAS BEEN RECEIVED BY NIH AND ASSIGNED TO AN INITIAL REVIEW GROUP (IRG) FOR SCIENTIFIC MERIT EVALUATION AND TO AN INSTITUTE FOR FUNDING CONSIDERATION. THE INITIAL PEER REVIEW SHOULD BE COMPLETED BY 12/90 AND A FUNDING DECISION SHOULD BE MADE SHORTLY AFTER THE APPROPRIATE NATIONAL ADVISORY GROUP MEETS IN 01/91. FOR QUESTIONS ABOUT THE ASSIGNMENT, CONTACT THE REFERRAL OFFICE (301) 496-7447. FOR QUESTIONS PRIOR TO THE REVIEW, CONTACT THE IRG EXECUTIVE SECRETARY OR THE INDIVIDUAL LISTED ABOVE. FOR QUESTIONS AFTER THE REVIEW, CONTACT THE INSTITUTE LISTED BELOW.

PRINCIPAL INVESTIGATOR: JOHNSON, LESLIE S COUNCIL: 01-91
TITLE: HUMAN-MURINE CHIMERIC ANTIBODIES AGAINST RESPIRATORY SYN
ASSIGNMENT NUMBER: 1R43AI30300-01A1 IRG: SSS 4

NATL INST ALLERGY/INFECTIOUS DIS
EXTRAMURAL ACTIVITIES RM 703
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MD 20892 (301)496-7291

VICE PRESIDENT
RESEARCH & DEVELOPMENT
MOLECULAR VACCINES, INC
19 FIRSTFIELD ROAD
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OMB No. 0925-0196
Expiration Date 12/31/00

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE

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TYPE ACTIVITY NUMBER

SMALL BUSINESS INNOVATION RESEARCH PROGRAM PHASE I GRANT APPLICATION

REVIEW GROUP FORMERLY

FOLLOW INSTRUCTIONS CAREFULLY

COUNCIL/BOARD (Month/year) DATE RECEIVED

1. TITLE OF APPLICATION (Do not exceed 56 typewriter spaces)
Human-Murine Chimeric Antibodies Against Respiratory Syncytial Virus

2. SBIR SOLICITATION NO. **PHS 90-2**

3. PRINCIPAL INVESTIGATOR

3a. NAME (Last, first, middle)
Johnson, Leslie Sydnor

☐ New Investigator

3b. SOCIAL SECURITY NO.
137-58-7149

3c. POSITION TITLE
Scientist

3d. MAILING ADDRESS (Street, city, state, ZIP code)
Molecular Vaccines, Inc.
19 Firstfield Road
Gaithersburg, MD 20878

3e. TELEPHONE (Area code, number and extension)
(301) 590-2622

4. HUMAN SUBJECTS
☒ NO ☐ YES
☐ Exemption # _____
OR
☐ Form HHS 596 enclosed

5. VERTEBRATE ANIMALS
☐ NO ☒ YES

6. DATES OF PROJECT PERIOD

7. COSTS REQUESTED

7a. Direct Costs
$ 24,246

7b. Total Costs
$ 50,303

8. PERFORMANCE SITES (Organizations and addresses)
Molecular Vaccines, Inc.
19 Firstfield Road
Gaithersburg, MD 20878

9. APPLICANT ORGANIZATION (Name, address, and congressional district)
Molecular Vaccines, Inc.
19 Firstfield Road
Gaithersburg, MD 20878

6th Congressional District

10. ENTITY IDENTIFICATION NUMBER
52-1555759

11. SMALL BUSINESS CERTIFICATION
☒ Small business
☐ Minority and disadvantaged
☐ Woman-owned

12. NOTICE OF PROPRIETARY INFORMATION
The information identified by asterisks (*) on pages _____ of this application constitutes trade secrets or information that is commercial or financial and confidential or privileged. It is furnished to the Government in confidence with the understanding that such information shall be used or disclosed only for evaluation of this application; provided that, if a grant is awarded as a result of or in connection with the submission of this application, the Government shall have the right to use or disclose the information herein to the extent provided by law. This restriction does not limit the Government's right to use the information if it is obtained without restriction from another source.

13. DISCLOSURE PERMISSION STATEMENT
If this application does not result in an award, is the Government permitted to disclose the title only of your proposed project, and the name, address and telephone number of the corporate official of your firm, to organizations that may be interested in contacting you for further information or possible investment?
☒ YES ☐ NO

14. CORPORATE OFFICIAL
(Name, title, address and telephone number)
Dr. James F. Young
Vice President, Research & Development
Molecular Vaccines, Inc.
19 Firstfield Road
Gaithersburg, MD 20878 Tel: (301) 590-2622

15. PRINCIPAL INVESTIGATOR ASSURANCE:
I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application. Willful provision of false information is a criminal offense (U.S. Code, Title 18, Section 1001).

SIGNATURE OF PERSON NAMED IN 3a
(In ink. "Per" signature not acceptable)

DATE

16. CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true and complete to the best of my knowledge, and accept the obligation to comply with Public Health Service terms and conditions if a grant is awarded as the result of this application. A willfully false certification is a criminal offense (U.S. Code, Title 18, Section 1001).

SIGNATURE OF PERSON NAMED IN 14.
(In ink. "Per" signature not acceptable)

DATE

RESEARCH PLAN

A. Specific Aims

Murine monoclonal antibodies against the F glycoprotein of RSV have been developed and characterized. These have been shown to neutralize RSV *in vitro* and protect cotton rats from lower respiratory tract infection *in vivo*. Previous studies with other rodent antibodies suggest that the development of human anti-mouse antibody responses would preclude the use of these murine antibodies directly in children. Therefore, it is the objective of this Phase I study to develop humanized versions of these anti-RSV antibodies which could then be used for passive immunization against RSV infection.

The specific objectives of Phase I work are as follows:

1. Clone and sequence the variable domains of the heavy and light chains from two selected murine anti-RSV monoclonal antibodies.
2. Substitute the coding regions for the complementarity determining regions from the murine cDNAs for the corresponding regions in human antibody heavy and light chain sequences.
3. Express the so constructed genes in mammalian cells and purify the humanized antibodies.
4. Characterize these antibodies with respect to:
   a) antigen binding compared to the mouse parent
   b) *in vitro* RSV neutralization, and
   c) *in vivo* protection against RSV challenge in the cotton rat model

The successful completion of the studies described in this application will provide the basis for producing a product which could be tested in humans for prophylaxis against RSV infections in Phase II studies. It would also be prototypical for the development of humanized antibodies against other infectious agents and their use in treatment or prevention of illness.

B. Significance

Respiratory syncytial virus (RSV) is the most common cause of lower respiratory tract infection in young children. In the United Sates alone, approximately 90,000 children are hospitalized and 4,500 children die from RSV infection each year (1). At special risk of serious RSV morbidity and mortality are children with underlying diseases such as congenital heart disease, bronchopulmonary dysplasia (BPD) and other pulmonary diseases, various congenital or acquired immunodeficiency syndromes and prematurity (2-5). There are over 100,000 such high risk children in the United States.

Infants with congenital heart disease (CHD) have a high fatality rate from RSV infection. In a prospective study, MacDonald et al (2) compared the course of hospitalized infants with CHD with and without RSV infection; the 27 CHD infants with RSV infection had a higher mortality rate (37%) both than the 46 CHD infants without RSV infection (6.5%) ($p<0.01$) and than the 202 infants without CHD but with RSV infection (1.5%) ($p<0.01$). In RSV infected infants with bronchopulmonary dysplasia, a chronic lung disease occurring in approximately 20% of all preterm infants requiring mechanical ventilation at birth (6), Groothuis et al (3) demonstrated a high degree of morbidity. Sixteen of 30 BPD patients followed prospectively over one winter were infected with RSV; 11 of them required hospitalization (4 in the intensive care unit). Although no fatalities occurred in this study, RSV is recognized as a common cause of death in BPD patients (7). Infants born

prematurely are a third and numerically important risk group for RSV infection, particularly during their first 6 months of life. In a nine year prospective study at the University of Rochester (5), 30% of children hospitalized with RSV pneumonia or bronchiolitis had been born prematurely. It is clear that RSV infection can be fatal in such infants (7).

Although an antiviral drug, ribavirin, has recently been licensed for therapy of RSV pneumonia and bronchiolitis (8,9), its value is controversial (10). Unfortunately, RSV vaccines are unavailable and unlikely to be licensed in the near future (1). A major obstacle to vaccine development is safety; the initial formalin inactivated RSV vaccine caused an increased incidence of RSV lower respiratory tract disease and death in immunized children (11). Currently the most promising approach to prophylaxis of RSV disease in high risk infants is passive immunization (12).

Support for the potential utility of passive immunization derives from studies of immune globulin or monoclonal antibodies in the prevention of experimental RSV infection in cotton rats (12-14) and Aotus monkeys (12, 15). In these animal models, immune globulin or monoclonal antibody infused prior to viral challenge prevented lower respiratory infection and reduced nasal RSV titers by 50-fold. Similar studies showed that high-titered RSV immunoglobulin was effective therapy for RSV infections in these animal models (13, 15). Infected animals treated with RSV immune globulin, showed no evidence of pulmonary immune-complex disease (13, 15). A double-blind, placebo-controlled intravenenous immunoglobulin (IVIG) immunotherapy study was undertaken in 35 children hospitalized with RSV infection (16). Therapy was well tolerated and resulted in significant reductions in nasal RSV shedding and oxygenation in the treated infants. An NIAID sponsored multi-center Phase I safety trial of intravenous immune globulin given prophylactically to high risk infants was recently completed (17). Infusion of 10-15 ml/Kg of commercial lots of IVIG selected for high neutralizing antibody titer was generally well tolerated. RSV infection was detected in 9 of 21 patients, only one of whom had severe illness. Molecular Vaccines, Inc. ("MVI") is developing an RSV intravenous hyperimmune globulin for the prevention and treatment of RSV disease in high risk children. In collaboration with Massachusetts Public Health Biologic Laboratories, plasma from donors was screened by a virus micro-neutralization assay to identify those containing high titers of neutralizing antibody to RSV. These were then pooled and used to prepare a hyperimmune globulin which is currently being tested in a NIAID-sponsored multicenter Phase II efficacy trial for the prophylaxis of RSV lower respiratory tract infection in infants with serious heart and lung disease.

Even if RSV hyperimmune globulin is shown to reduce the incidence and severity of RSV lower respiratory tract infection in high risk children, several disadvantages may limit its use. One drawback is the necessity for intravenous infusion in these children who have limited venous access because of prior intensive therapy. A second disadvantage is the large volume of RSVIG required for protection, particularly since most these children have compromised cardiopulmonary function. A third disadvantage is that intravenous infusion necessitates monthly hospital visits during the RSV season which places these children at risk of nosocomial RSV infection (7). A final problem is that it may prove impossible to select sufficient donors to produce a hyperimmune globulin for RSV to meet the demand for this product. Currently only about 5% of normal donors have RSV neutralizing antibody titers high enough to qualify for the production of hyperimmune globulin.

One approach to circumventing a number of these problems may be to vaccinate adult volunteers with a live RSV vaccine to boost natural immunity in these individuals. This may then increase the number of individuals who have serum antibody titers high enough to be used to prepare a hyperimmune globulin, or to increase titers sufficiently high to prepare a product which could be used intramuscularly.

Another approach may be the development of monoclonal antibodies with high specific neutralizing activity as an alternative to hyperimmune globulin. The advantages of a monoclonal antibody approach are several, the most significant of which is that it would permit the production of a highly defined product with a standardized potency. Unlike an immunoglobulin product in which only a small fraction of the antibody is RSV-specific, a monoclonal antibody product would not contain any irrelevant antibody. Hence, the specific activity would be 50- to 100- fold higher than an immune globulin product and could potentially be used intramuscularly. In addition, if recombinant DNA methodology is used for production of the monoclonal antibody, it can be selectively engineered to provide the desired biological properties (F_c effector function, valency, pharmacokinetics, etc.) Monoclonal antibodies to other infectious agents for which these children are at risk (influenza and parainfluenza viruses) could also be combined to produce a "cocktail" and provide broader protection to these children. Since it is likely that repeated dosing of these monoclonal antibodies will be required during the high risk RSV season (November to March), it will be preferable, if not necessary, to use human monoclonal antibodies rather than murine or rat antibodies to minimize the development of human anti-rodent antibody responses which may compromise the therapeutic efficacy of the antibody or induce immune-complex pathology. However, the generation of human monoclonal antibodies with the desired specificity may be difficult and the level of production from human cell lines is often low, precluding their development.

An alternative is to produce human-mouse chimeric antibodies which contain the binding specificity of a pre-characterized murine antibody. A series of eighteen murine monoclonal antibodies against the RSV F glycoprotein have already been produced (18). They have been characterized with regard to their neutralizing potential, both to the parental RSV strains used to immunize the mice to generate the hybridomas, as well as a number of different virus strains isolated from 1956 to 1985 from various geographical locations. They have also been shown to provide protection in the cotton rat challenge model (Dr.Greg Prince, personal communication). In addition, these antibodies have been mapped by competitive binding and reactivity profiles of virus escape mutants to three broad antigenic sites (A, B and C) containing 16 epitopes. Epitopes within antigenic sites A and C show the least variability in natural isolates. MVI has obtained through Research Materials Transfer Agreements with Dr. Brian Murphy, National Institutes of Health and Dr. Larry Anderson, Centers for Disease Control, hybridomas producing eight of these murine monoclonal antibodies for further studies. These antibodies should were selected because they represented seven different epitopes, reacted with the highest number of virus strains tested (at least 13 out of 14 isolates) and retained the greatest neutralizing activity against virus escape mutants selected with the other antibodies. Table I summarizes the features of these eight antibodies.

Table I.
Characteristics of murine monoclonal antibodies (31)

Monoclonal Antibody	Antigenic site	Epitope	Immuno-globulin subclass	# of RSV strains neutralized
1153	A	1	IgG_1k	13/14
1142	A	1	IgG_1k	13/14
1129	A	4	IgG_1k	13/14
1121	A	5	IgG_1k	13/14
1243	C	11	$IgG_{2A}k$	13/14
1332H	C	12	$IgG_{2A}k$	14/14
1308F	C	13	IgG_1k	14/14
1302A	C	14	IgG_1k	14/14

Protection Against RSV Proliferation, *in vivo*

We examined eight of these monoclonal antibodies, specific for the RSV F glycoprotein, for their ability to block RSV replication when administered to cotton rats by the intranasal route prior to virus challenge. These antibodies were selected because they represented seven different epitopes, reacted with the highest number of virus strains tested (at least 13 of 14 isolates) and retained the greatest neutralizing activity against virus escape mutants selected with the other antibodies. Table I summarizes the features of these eight antibodies.

Cotton rats (*S. fulviventer*, 3 animals per group, average weight 100 grams) were anesthetized with methoxyflurane and given 0.1 ml of antibody solution (10 mg/ml or 1 mg/ml in phosphate-buffered saline (PBS) by intranasal (i.n.) instillation. A pooled monoclonal antibody mixture of all eight monoclonal antibodies (10 mg/ml total, 1.25 mg/ml each or 1 mg/ml total, 0.125 mg/ml each) was tested. Control animals were given 0.1 ml of a bovine serum albumin suspension (10 mg/ml or 1mg/ml) in PBS or standard human immune globulin (Sandoglobulin, Sandoz, Inc., East Hanover, N.J.) in PBS (10 mg/ml or 1 mg/ml). One day later, animals were again anesthetized with methoxyflurane and challenged by i.n. instillation of 10^5 plaque forming units (PFU) of the Long strain of RSV. Four days after virus challenge, all animals were sacrificed by carbon dioxide asphyxiation. Lungs were harvested and homogenized in 10 parts (wt/vol) of Hanks balanced salt solution supplemented with 0.218 M sucrose, 4.4 mM glutamate, 3.8 mM KH2PO4 and 3.2 mM K2HPO4 and the resulting suspension was stored at -70°C until assayed for virus content. Virus titers were determined using a plaque assay on HEp-2 cell monolayers and are expressed as PFU per gram of tissue.

The results shown in figure 1 indicate that each of the monoclonal antibodies showed a significant reduction in pulmonary virus titer, and were at least as effective and in most cases more effective than the standard immune globulin or the pool of the eight monoclonal antibodies. Surprisingly, five of the eight monoclonal antibodies appear to completely prevent infection at the high dose and one even at the low dose.

The eight MAbs described above were further examined for their ability to block RSV replication when administered at lower doses to cotton rats by the intranasal route prior to virus challenge. Cotton rats (*S. hispidus*, 4 animals per group, average weight 100 grams) were anesthetized with methoxyflurane and given 0.1 ml of antibody solution (1 mg/ml, 0.1 mg/ml or 0.01 mg/ml in phosphate-buffered saline (PBS) by intranasal (i.n.) instillation. Control animals were given 0.1 ml of a bovine serum albumin suspension (1 mg/ml) in PBS. One day later, animals were again anesthetized with methoxyflurane and challenged by i.n. instillation of $10^{5.0}$ plaque forming units (PFU) of the Long strain of RSV. Four days after virus challenge, all animals were sacrificed by carbon dioxide asphyxiation. Lungs were harvested and homogenized in 10 parts (wt/vol) of Hanks balanced salt solution supplemented with 0.218 M sucrose, 4.4 mM glutamate, 3.8 mM KH_2PO_4 and 3.2 mM K_2HPO_4 and the resulting suspension was stored at -70°C until assayed for virus content. Figure 4 shows that each of the monoclonal antibodies, except MAb 1243 showed a significant reduction in pulmonary virus titer when given at the 0.1 mg or 1 mg doses. MAb 1308 showed the best reduction among those to the C region of the F protein, while 1129 and 1121 were the best among those which bind to the A region.

Figure 1

Prophylaxis of RSV Infection in S. Fulviventer Using Anti-F Monoclonal Antibodies

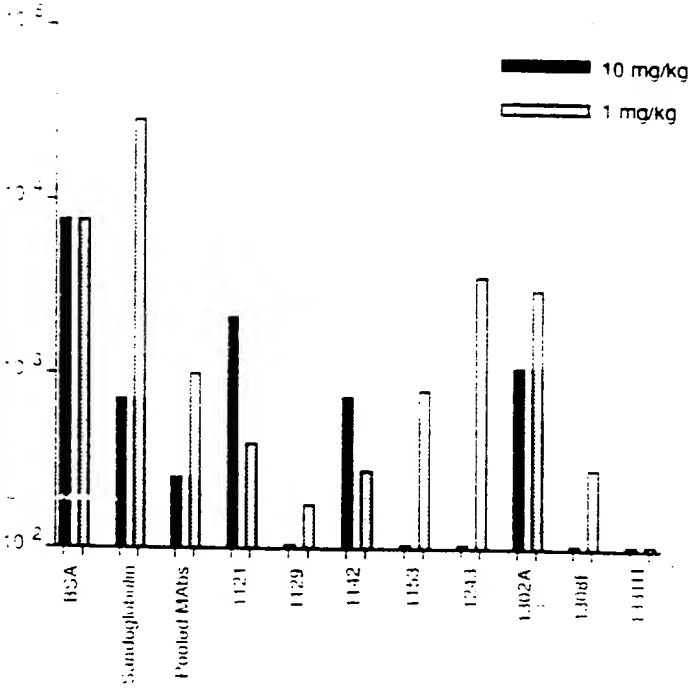


Figure 2

Prophylaxis of RSV Infection in S. Hispidus Using Anti-F Monoclonal Antibodies

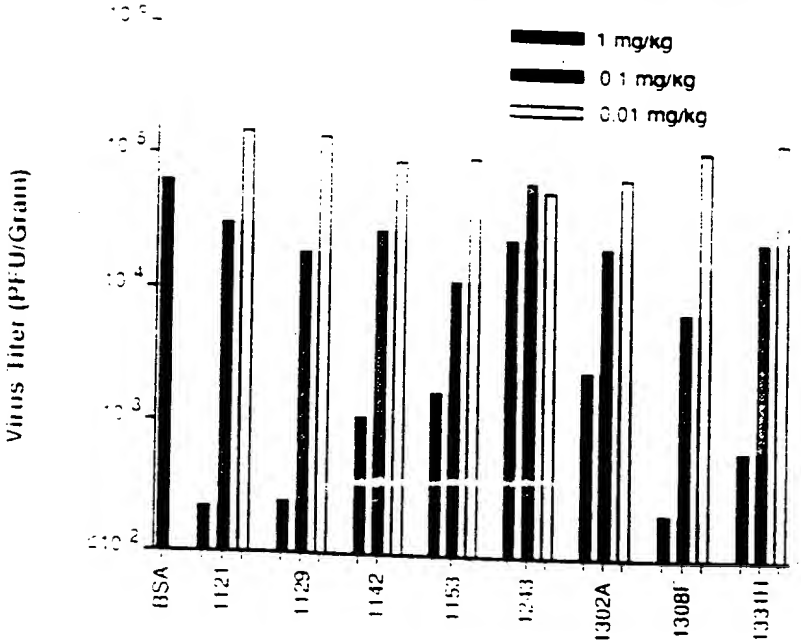


Figure 3

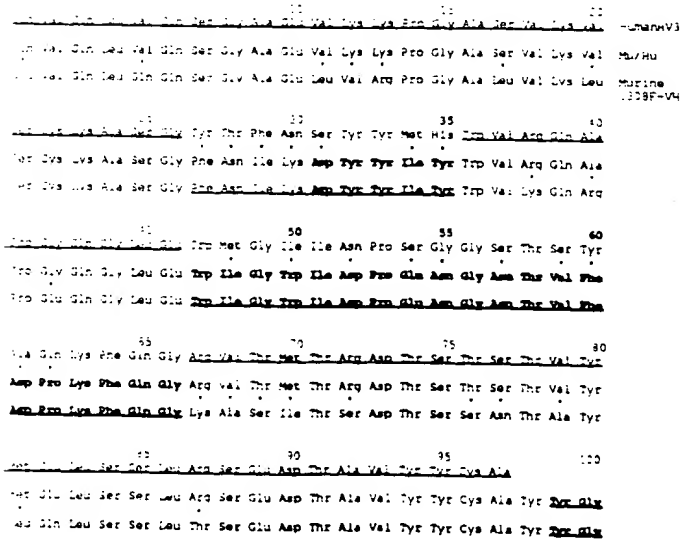
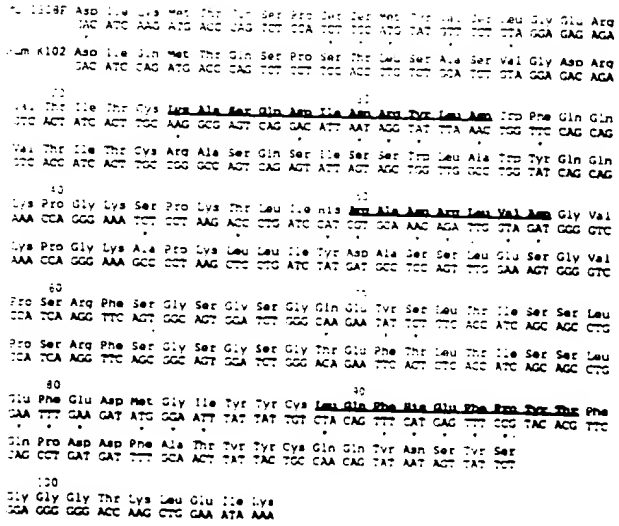


Figure 4



RSV Neutralization *in vitro*

The eight monoclonal antibodies were also tested for their ability to neutralize virus in vitro. Neutralization was measured by a plaque-reduction assay The data are presented in Table 2. All antibodies showed neutralizing activity at the high concentration (10 mg/ml) whereas three of the monoclonal antibodies (1243,1302A and 1331H) showed no activity at the lower concentration (1 mg/ml).

While there appears to be a correlatation between *in vitro* neutralizing activity and *in vivo* protection for the antibodies to the C region of the F protein, such a correlation is not apparent for those to the A region.

Table II.
Neutralization Titers for Monoclonal Antibodies

	10 mg/ml	1 mg/ml
BSA	<20	<20
Sandoglobulin	1360	200
Pooled MAbs	4100	300
1121	1340	120
1129	3540	240
1142	6140	420
1153	9940	700
1243	300	<20
1302A	480	<20
1308F	3540	440
1331H	1960	<20

Currently the murine antibodies are being further tested individually, and in pairwise combinations in the cotton rat for protective efficacy and potential synergy or competition. Two of these antibodies will then be selected, most probably one from antigenic site A and one from antigenic site C, to produce mouse-human chimeric antibodies. Although a number of laboratories have constructed chimeric antibodies with mouse variable and human constant domains (19-27), the mouse variable region may still be seen as foreign (28). Therefore, it is preferable to construct humanized antibodies in which only the hypervariable or complementarity determining regions (CDRs) of the heavy and light chains from the murine antibody of the desired specificity are transplanted onto the framework of a human antibody (28-30). These antibodies retain the binding capacity and affinity of the murine monoclonal antibody. Since the CDRs do not contain characteristic rodent or human motifs, these humanized antibodies should be indistinguishable in sequence from completely human antibodies thus minimizing human antibody responses. Once the humanized antibodies have been produced and characterized in these Phase I studies, Phase II studies would be initiated to examine the protective efficacy of these molecules in clinical studies.

Cloning and Analysis of V-region cDNA's

On the basis of the above data, we selected the antibodies 1308F, specific for the C region of the RSV F protein, and 1129, specific for the A region as the initial targets for humanization. Total RNA from 1308F hybridoma cells was purified by the method of Chirgwin and subjected to one round of oligo dT chromatography. Double stranded cDNA was made from this RNA using primers complementary to the g1 or K constant region segment. The cDNA was inserted into pUC18 at the Sma I site and transformants were screened using a second oligonucleotide from a region of the c-region between the first strand cDNA primer and the variable region. cDNA inserts from positive clones were subjected to DNA sequencing and the variable region sequences were determined.

A comparison of the 1308F V_H sequence with all sequences in the NBRF protein sequence database was performed using the wordsearch program from the University of Wisconsin analysis programs. This search revealed significant homology to two human V_H regions. The highest homology was to the human germline gene HV3. Thetwo sequences are 62% homologous overall and 65% in the framework regions. Significantly there is good homology at the junctions of the CDR segments and the frameworks with the exception of the 5' end of FR2. The mouse and human sequences as well as that of a potential CDR-Grafted combination of the two is shown in figure 3.

A similar analysis of the VL region revealed high homology to the human germ line V-kappa gene K102. The alignment of these sequences is shown in figure 2. In this case the homology is 62% overall and 73% in the framework regions. In each case a human J-region can be selected which is identical to the mouse sequence. This comparison is shown in figure 4.

C. Relevant Experience

L. Syd Johnson (Ph.D.) is a molecular biologist with considerable experiance in the cloning, sequencing, analysis and expression of antibody segments. While at Genex Corporation he was instrumental in the development of single-chain antibody technology.

Geetha Bansal (Ph.D.) has more than 10 years experience in the production of monoclonal antibodies, and in Western blotting, RIA,ELISA, receptor binding, T cell proliferation and lymphokine assays. She is currently involved in the development of parvovirus B19 diagnostic assays, vaccines and hyperimmune globulin as well as AIDS vaccine and AIDS immunotherapeutic research and development.

David Feller (Ph.D.) is a biological computational chemist with experience in writing and using computer applications for the purpose of structure-function analysis of proteins and nucleic acids.

Molecular Vaccines, Inc. is currently developing and testing hyperimmune globulin products for three human virus infections: respiratory syncytial virus (RSV), Parvovirus B19 and cytomegalovirus (CMV). Further, we have research, development and business relationships in place withMassachusetts Public Health Biologic Laboratories (Dr.George Siber) for production of these immunotherapeutic products. Clinical trials are in their second year for the RSV product. The CMV immunotherapeutic has been approved by the FDA an is expected to be marketed in

D. Experimental Design and Methods

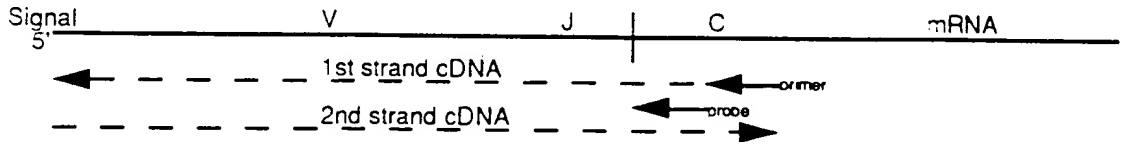
To develop humanized antibodies against RSV, we will begin with murine hybridoma cells producing murine monoclonal antibodies which have been shown to neutralize RSV *in vitro* and protect cotton rats against lower respiratory tract infection with RSV. RNA will be extracted from these cells, the rearranged immunoglobulin variable regions will be cloned and sequenced and murine CDR's will be substituted into human heavy and light chain cDNAs. The humanized antibodies will then be expressed and characterized relative to the parental murine antibodies to be certain that the genetic manipulation has not drastically altered the binding properties of the antibodies.

1. Cell Growth and mRNA Extraction

Murine Hybridoma cell lines will be maintained in RPMI 1640 supplemented with 15% fetal bovine serum. Cell culture supernatants will be routinely assayed by ELISA for the production of antibody with specificity for the RSV F protein. Total RNA from 1 x 10^8 cells will be extracted by the method of Chirgwin, *et al* (32). pA^+ RNA will be purified by oligo-dT chromatography using spin columns. We have also had success purifying pA^+ RNA adequate for cDNA cloning of immunoglobulin variable regions using the Fast-Track kit from Invitrogen.

2. Variable Region cDNA Cloning and Sequencing

cDNA copies of the VH and VL of the target antibody will be generated essentially as described by Bedzyk, *et al* (33). The first strand cDNA reaction will be carried out using a phosphorylated oligonucletide complementary to a segment of the mRNA coding for the constant region of the particular heavy or light chain isotype. This primer anneals to a segment of the mRNA adjacent to the variable region. Second strand cDNA synthesis will be carried out using RNase H and E. coli DNA polymerase I, as described by Gubler and Hoffman (34), followed by T4 DNA polymerase to assure that blunt ends are produced.



The ds-cDNA will then be ligated into pUC18 which has been digested with SmaI and treated with alkaline phosphatase. The ligated DNA will be used to transform E. coli DH5a by the method of Hanahan (35). Colony hybridization will be used to identify transformants carrying the desired cDNA segment. The probe for the hybridization will be a second segment of the c-region sequence lying between the first strand cDNA primer and the V-region. Probes corresponding to the J segments of the variable regions can also be used. Insert size of the plasmids in the positive clones will be determined. Because the 5' end of the first strand cDNA is fixed a nested set of sequences can be chosen for sequencing by picking those approximately 450bp (full length), 300bp and 150bp, subcloning into M13mp18 and mp19 and sequencing. After the sequence is determined in this way full length clones will be sequenced entirely on both strands to assure that this agrees with the composite sequence.

By taking advantage of the fact that immunoglobulin variable region genes are flanked by conserved sequences on one end and by semi-conserved sequences at the other, it is possible to design consensus primers to selectively clone the variable regions of rearranged immunoglobulin genes by the use of the polymerase chain reaction (PCR) (36,37). This approach will be used as an alternative if the cDNA cloning does not proceed rapidly, and as a replacement for this step at a later time.

3. Design and Assembly of Humanized Antibodies

The CDR regions of the V_H and V_L will be identified by comparing the amino acid sequence to known sequences as described by Kabat (38). In order to select the human framework sequences best able to accept the mouse derived CDR sequences in a conformation which retains the structure of the antigen combining site, the following strategy will be employed. First, the sequence of the murine V_H region will be compared to known human sequences from both the Genbank and NBRF protein databanks using the Wordsearch program in the Wisconsin package of sequence manipulation programs (39). The best several human V-regions will then be

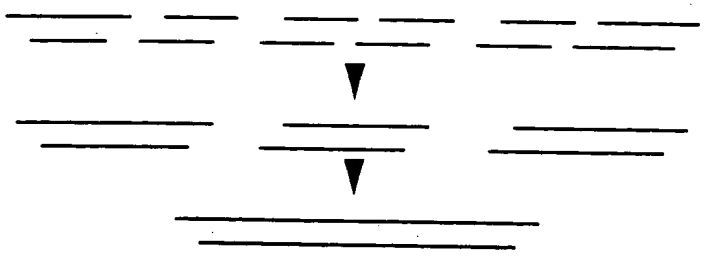
analyzed further on the basis of similarity in the framework regions, especially at the junctions of the framework and CDR regions.

Molecular modeling will be done using Silicon Graphics (SGI) computers at United States Army Research Institute of Infectious Diseases (USAMRIID) and at the Army's Ballistics Research Laboratory (BRL). The computer application that will be used is DISCOVER (Biosym Technologies, San Degio, CA) which is built upon the AMBER potential energy functions.

The human V_H regions selected in this way and the original mouse sequences will be modeled onto the corresponding region of another antibody for which a high resolution X-ray structure is available, such as MCPC603. The murine CDR regions will then be inserted into the human model and the structures compared with particular attention to (a) the structure of the combining site and (b) possible perturbations of the framework structure. In cases where there is a divergence in the mouse and human sequences at the boundaries of a CDR both will be modeled. In general preference will be given to retaining the mouse residues in such a case if it appears likely that substitution of the human residues will perturb the CDR structure.

The human V_L region can be selected in one of two ways (a) Use the V_L which was associated with the human V_H used or (b) use the same strategy as was employed to select the human V_H framework. Which approach we use will depend on the availability of a both V_L and V_H sequences in the databases and relative homology of the two to the murine counterparts. The former approach is preferable if both sequences are available. In a case where a human V_H framework is found and the V_L is not available, such as where the human sequence is from an unrearranged germline gene, the latter strategy will be undertaken. As long as a V_L of the same subgroup as the one chosen has been found with a similar V_H such an approach should be valid.

The designed variable region genes will be assembled from oligonucleotides. Complementary pairs of 60mers with 10 base pair overlap will be made and annealed. Adjacent gene segments will be ligated together pairwise and then three sets will be combined to assemble an intact VL or VH gene segment (350bp).



4. **Expression of Humanized Antibodies**

Once the humanized VL and VH have been designed and modeled the project becomes more empirical in nature. Genes will be designed synthesized and expressed, at which time the question of whether the affinity of the recombinant antibody is similar to the murine Mab from which is was derived can be answered. The rapidity with which these steps can be undertaken is thus of importance in finally obtaining a humanized antibody which retains most of the affinity of the parent. To this end we will work with an transient expression systems to produce large enough amounts of antibody or antibody fragments to compare to the native antibody or fragments therefrom.

The ability of recombinant vaccinia virus carrying the bacteriophage T7 RNA polymerase gene to promote efficient expression of foreign genes co-introduced under the control of a T7 promoter will be utilized as our initial production system. Plasmid vectors containing a T7 promoter and a Cap-independent translation initiation signal from the encephalomyocarditis (EMC) virus are available from Dr Thomas Fuerst at MVI. We have recently used such a vector to produce an active single-chain antibody (SCA) at a level of approximately 2ug/ml. The active SCA appeared to have been secreted as evidenced by removal of the signal peptide. While we could also produce an SCA version of the chimeric antibody, we do not foresee the necessity of this step for this project, where the end product will be a full sized antibody. Instead we will co-express heavy and light chain genes either on the same plasmid or introduced on different plasmids. Production of whole immunoglobulins from a single recombinant virus has been achieved in insect cells using a baculovirus system (43). The utility of a rapid transient system depends on how much "tinkering" is required on the recombinant protein(s). If the initial design produces an antibody with acceptible binding and neutrallization characteristics it could go directly into the final expression system. If, on the other hand, more modifications need to be made the utility of the transient expression system will be significant.

Stably co-transformed mouse myeloma cell lines or "transfectomas" will then be generated in order to provide an ultimate production source for the modified antibodies. This will be done in a manner similar to that described by Oi, *et al.* (44). The heavy and light chain hybrid human-mouse cDNAs will be separately inserted into different expression vectors containing Ig promoter/enhancer sequences, to drive transcription of the cDNAs (22,29-31), and the dominant selectable markers for G418 (neo) or mycophenolic acid (gpt).(22). The expression vectors for the heavy and light chain cDNAs will then be co-transfected (electroporated) into Sp2/0, a non-producing mouse myeloma cell line. Transfectants will be selected initially for G418 resistance; stable transfectants will then be selected in medium containing both G418 and mycophenolic acid to derive cell lines which have incorporated vectors for both the heavy and light chains.

Cell lines will then be screened for production of heavy and light chain proteins by radioimmunoprecipitation. Cell lines expressing both chains will then be expanded, supernatants will be collected and antibody will be purified by affinity chromatography on protein G (Gammabind plus, Genex, Inc.).

5. **Characterization of Humanized Antibodies.**

a. Antibody binding to the RSV F protein. The purified antibodies will be assayed by an ELISA for reactivity with the RSV F glycoprotein expressed by a recombinant vaccinia virus (18) or purified RSV virions. To evaluate the relative binding affinity of the chimeric antibodies as compared to the parental murine antibody, competition solid-phase radioimmunoassays will performed (18). One antibody will be metabolically labeled with ^{35}S-methionine. A saturating amount of labeled antibody will be mixed with increasing concentrations of either the murine or chimeric antibodies and competition curves generated. Reciprocal competition experiments will also be performed. In this way inhibition curves can be compared and the relative binding affinity of the antibodies can be determined. This assay is in place at MVI and has been used to map MAb's to different antigenic sites.

b. *In vitro* virus neutralization. Virus neutralization curves will be determined in a standard assay. Serial two-fold dilutions of antibodies will be mixed with 100 tissue culture infectious dose$_{50}$ of RSV in the presence of 5% guinea pig complement. After

sixty minutes, the virus antibody mixtures will be transferred to Hep-2 cells in 96-well plates and six days later monolayers will be examined microscopically for cytopathology. Neutralization titers will be expressed as the lowest antibody concentration which inhibits more than 95% of viral cytopathic effect present in control wells.

c. *In vitro* protection studies in cotton rats. Cotton rats will be administered different doses of antibody 24 hours prior to intranasal challenge with RSV. Four days after challenge the animals will be sacrificed and viral titers in lung and nasal tissues will be determined by a standard plaque assay on Hep-2 cell monolayers as described (13). These studies will be done through a contractual arrangement with Jackson Foundation (see section H).

E. Human Subjects

None

F. Vertebrate Animals

The proposed project involves the use of adult cotton rats (*Sigmodon hispidus*) to assess the protective efficacy of antibodies against RSV challenge. Animals will be obtained from the Veterinary Resources Branch, Division of Research Services, National Institutes of Health. Approximately 45 animals will be used for these studies.

Animals will be housed at the Uniformed Services University of the Health Sciences or off-site at a contract facility on Medical Center Drive, in Gaithersburg, Maryland. An AALAC certified veterinarian is available to counsel of the care and use of animals. Veterinary care includes a program for prevention of disease; daily observation and surveillance for assessment of animals health; appropriate methods of disease control, diagnosis, and treatment; and guidance of animal users in appropriate methods of handling, restraint, anesthesia, and euthanasia.

All procedures in this project ensure minimal discomfort to animals. Animals will be anesthetized with methoxyflurane prior to intranasal instillation of antibody and virus challenge. At four days post-infection; animals will be sacrified by carbon dioxide inhalation.

G. Consultants/Collaborators

(see section H).

H. Contractual Arrangements

MVI has established a collaborative research and development agreement with the Henry M. Jackson Foundation to develop and produce immunotherapeutic products to prevent or to treat serious respiratory infections caused by respiratory syncytial virus, parainfluenza viruses, and influenza. Participating from the Jackson Foundation are Drs. Val Hemming, Gerald Fisher, and Greg Prince who have pioneered the use of immunoglobulins in the therapy of respiratory syncytial virus infection. While the initial goal of the agreement is to develop high titered, enriched RSV immunoglobulin for intravenous use, second generation products are under development that are an improvement over intravenous immunoglobulin in regard to potency, duration/protection,

and ease of delivery. In addition, MVI has licensed from the U.S. Government exclusive rights for the topical (aerosolized) use of enhanced immune globulin for the therapeutic and prophylactic treatment of RSV infection. No support for this arrangement are sought though this SBIR proposal.

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44 Oi, V.T., Morrison, S.L., Herzenberg, L.A. and Berg, P. Proc. Nat. Acad. USA 80;825, 1983.

Other Grant and contract support

A. Active Research Support:

1. NIAID, NCVDG; Contract No. AIDS-1,1U01 AI28171-01
"Vaccines Development for Human Immunodeficiency Viruses"
P.I.: Wayne T. Hockmeyer
% effort, G. Bansal: 40%
D. Feller: 25%
Annual direct costs: $592,261
Grant period: 3/89-2/94

2. DOD, USAMRDC; log No. 89166005
"Flarvivirus RNA Replication: Essential Viral Functions as Targets for Antiviral Therapeutics"
P.I.: Marc S. Collett
%effort, G. Bansal: 10%
D. Feller: 30%
Annual direct costs: $216,633

B. Proposals Pending Funding:

1. NIH, Phase I SBIR
"Surrogate assay for B19 Parvovirus Serum Neutralization"
P.I.: Geetha Bansal: 30% effort
% effort, J. Hatfield: 20%
Annual direct costs: $24,172

D. Application Organization Active/Pending Funding for Work Related to this Project:

None

Figure 1

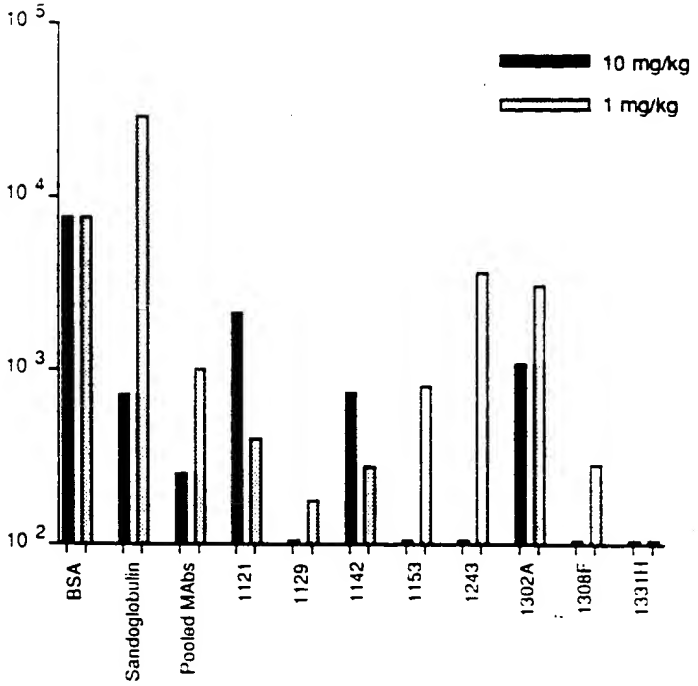


Figure 2

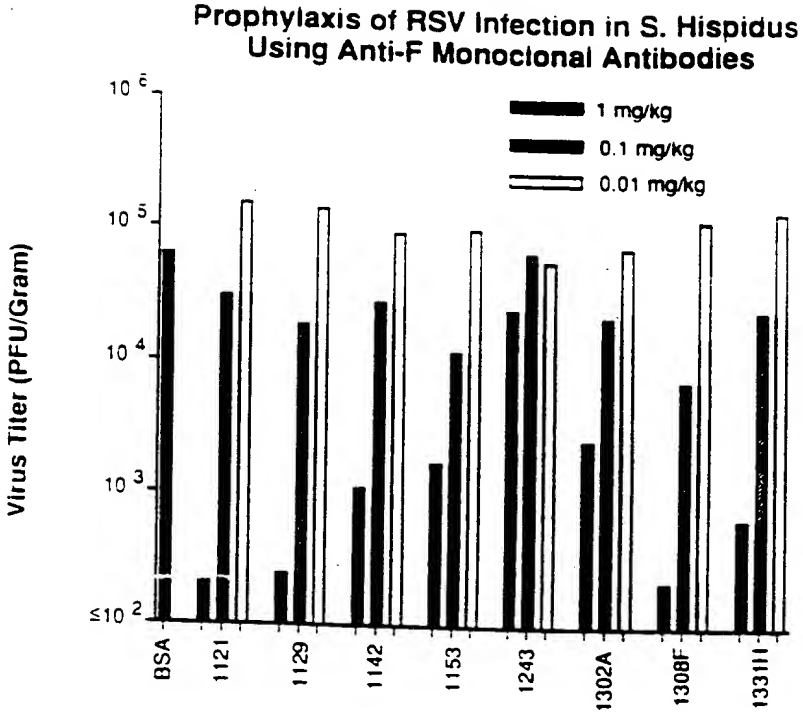


Figure 3

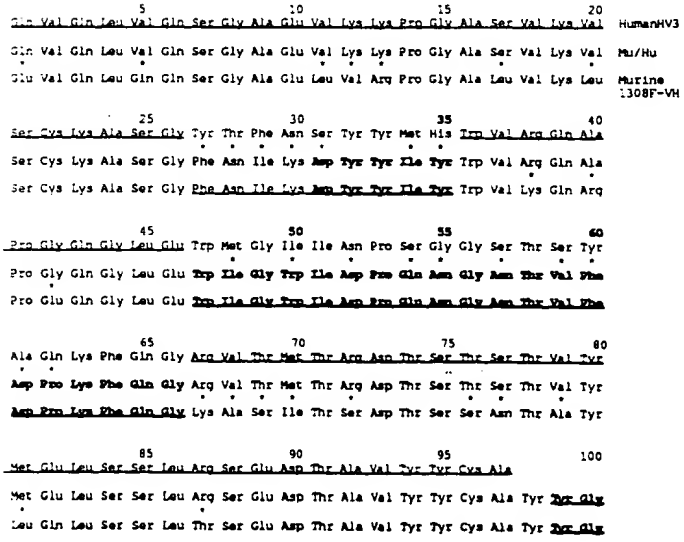
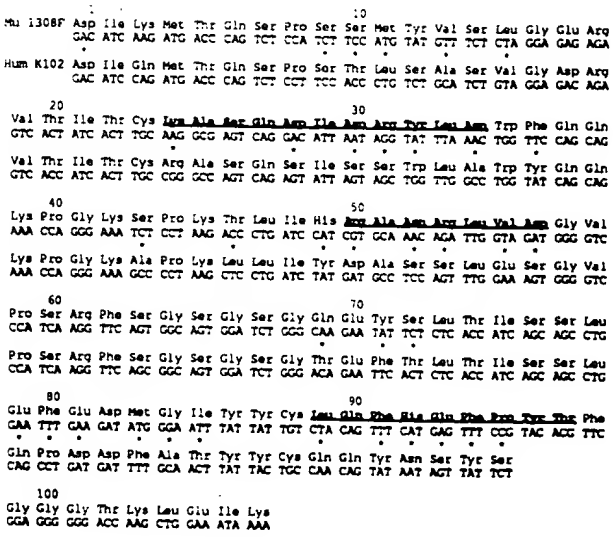


Figure 4



PRINCIPAL INVESTIGATOR: Johnson, Leslie Sydnor

CHECKLIST *(Front)*

This is the required last page of the application.

(Check the appropriate boxes and provide the information requested.)

TYPE OF APPLICATION

__ NEW application *(This application is being submitted to the PHS for the first time.)*

X REVISION of previously submitted application no. 1R43AI30300-01
(This application replaces a prior unfunded version of a new application.)

X Change of Principal Investigator *(If applicable)*
Name of Former Principal Investigator Young, James Francis

ASSURANCES *(See appendix to instructions.)*

a. Civil Rights	b. Handicapped Individuals	c. Vertebrate Animals *(If applicable)*	d. Human Subjects *(If applicable)*
X Filed	X Filed	X Filed	X Filed
__ Not Filed	__ Not Filed	__ Not Filed	__ Not Filed

ADDITIONAL ASSURANCES

The following additional certifications described below are made by checking the appropriate boxes and verified by th signature of the OFFICIAL SIGNING FOR APPLICANT ORGANIZATION on the FACE PAGE of the application.

f. **Delinquent Federal Debt.** [X] No [ ] Yes *(If "Yes," attach explanation.)*

Before a grant award can be made, the applicant organization must certify that it is **not** delinquent on the repayment of any Federal debt. The certification applies to the applicant organization, **not** to the person signing the application as the authorized representative **nor** to the principal investigator.

Examples of Federal debt include delinquent taxes, audit disallowances, guaranteed or direct student loans, FHA loans, business loans, and other miscellaneous administrative debts. For purposes of this certification, the following definitions of "delinquency" apply:

- For **direct loans** (whether awarded directly to the applicant by the Federal Government or by an institution using Federal funds), a debt more than 31 days past due on a scheduled payment.
- For **guaranteed and insured loans**, recipients of a loan guaranteed by the Federal Government that the Federal Government has repurchased from a lender because the borrower breached the loan agreement and is in default.
- For **grants**, organizations in receipt of "Notice of Grants Cost Disallowance" which have not repaid the disallowed amount or which have not resolved the disallowance. (Definition **excludes** cost disallowances in an "appeal" status.)

Where the applicant discloses delinquency on debt to the Federal Government, the PHS shall (1) take such information into account when determining whether the prospective grantee organization is responsible with respect to that grant, and (2) consider not making the grant until payment is made or satisfactory arrangements are made with the agency to whom the debt is owed. Therefore, it may be necessary for the PHS to contact the applicant before a grant can be made to confirm the status of the debt and ascertain the payment arrangements for its liquidation. Applicants that fail to liquidate indebtedness to the Federal Government in a businesslike manner place themselves at risk of not receiving financial assistance from the PHS.

g. **Debarment and Suspension.** [X] No [ ] Yes *(If "Yes," attach explanation.)*

Before a grant award can be made, the applicant organization must certify, among other things, that neither it nor its principals are presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from covered transactions by any Federal department or agency. Subawardees, that is, other corporations, partnerships, or other legal entities (called "lower tier" participants), must make the same certification to the applicant organization concerning their covered transactions. Please refer to the pertinent DHHS implementing regulations, Title 45 Code of Federal Regulations Part 76, for complete certification requirements.

Exhibit 2

TITLE _____ Project No. _____ Book No. _____

From Page No. _____

Sequence (Triplets): CAC GTC GAC ATT CAG CTG ACC
SalI PvuII
CAG TCT CCA _____
general V_L 5'
SJ41 conc. = 2.15 mg/ml

Sequence (Triplets): AGC GGA TCC AGG GGC CAG TGG
BamHI
ATA GAC _____
3' Mouse Gamma primer w BamHI site (Heavy ~~Light~~ chain)
SJ10 conc. : 1.247 mg/ml

Sequence (Triplets): CGG AAT TCA GGT TTA TCT GCA
RI PstI
GTA GTC W GG
[A T]
general V_H 5' SJ42 conc. = 2.66 mg/ml

Sequence (Triplets): GAT GGA TCC AGT TGG TGC AGC
Bam
ATC _____
3' mouse kappa primer with BamHI site (light chain)
SJ11 conc. = 2.402 mg/ml

To Page No. _____

Witnessed & Understood by me, Robert G. Bennett Date

Invented by R.J. Bennett Date 1/22/91

Recorded by

Project No.__ __

Book No.____ TITLE ____

Page No.__

SJ41 : $(330)(30) = 9900$

$(9900)^{-1}(2.15 \times 10^{-3}\ gm/ml) = 2.17 \times 10^{-7}\ mol/ml = 217\ pmol/\lambda$

$(217)x = (25)(1000)$

$x = 115.2\ \mu l$ per ml.

SJ10 : $(330)(27) = 8910$

$(8910)^{-1}(1.247 \times 10^{-3}) = 1.40 \times 10^{-7}\ mol/ml = 140\ pmol/\lambda$

$140x = (25)(1000)$

$x = 178.4\ \mu l$ per ml

SJ42 : $(330)(30) = 9900$

$(9900)^{-1}(2.66 \times 10^{-3}\ gm/ml) = 2.69 \times 10^{-7}\ mol/ml = 269\ pmol/\lambda$

$269x = (25)(1000)$

$x = 92.9\ \mu l$ per ml

SJ11 : $(330)(24) = 7920$

$(7920)^{-1}(2.4 \times 10^{-3}) = 3.03 \times 10^{-7}\ mol/ml = 303\ pmol/\lambda$

$303x = (25)(1000)$

$x = 82.5\ \mu l$ per ml

To Page No.____

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		Recorded by	

111

TITLE _____ Project No. _____ Book No. _____

From Page No. _____ PCR Reaction to generate V_L and V_H fragments from A region Mab 1129

1-22-91

Tube	Fragment	Template	Primers	Program
A	V_L 1129 Bam-Sal	1129 cDNA	SJ41	94°C 2 min
			SJ11	94°C 1 min
				55°C 2 min } 30 cy
B	V_H 1129 Eco-Bam	1129 cDNA	SJ42	72°C 2 min }
			SJ10	
				8°C soak o/N

1µl 1129 cDNA + 19µl H_2O

10µl 10µl

4µl 5' primer
4µl 3' primer
10µl 10 x PCR
10µl 10x dNTP
61µl H_2O
1µl AmpliTaq

A- V_L 1129
B- V_H 1129
C- Sid's PCR reaction

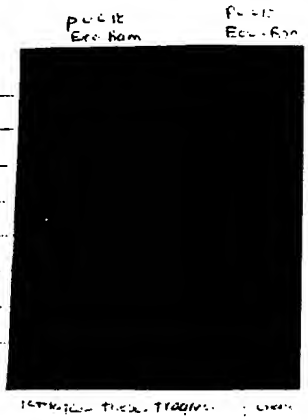
1-23-91

•phenol/chloroform ext, chloroform ext, ethanol ppt, 70% ethanol wash, resuspend in 36µl TE

•digestion V_L 1129 PCR fragment: 36µl DNA
4µl React 3
2µl SalI
2µl BamHI
Incubate 1.0 hr at 37°C

•digestion of V_H 1129 PCR fragment: 36µl DNA
4µl React 3
2µl EcoRI
2µl BamHI
Incubate 1 hr at 37°C

•digestion of pUC 18: 10µl DNA
8µl TE
4µl React 3
1µl enzyme: BamHI + EcoRI or BamHI + SalI



Isolate these fragments

To Page

Witnessed & Understood by me.	Date	Invented by S. Jewett	Date 1/22/91
		Recorded by	

Project No ____
Book No.____ TITLE ____

om Page No. 3

Ligation Reactions:

	pUC 18 Sal-Bam control	pUC18 EcoRI-Bam control	pUC18 V_L1129	pUC18 H_H1129
UC 18 Sal-BamHI f.	3µl	–	3µl	–
UC 18 EcoRI-BamHI f.	–	3µl	–	3µl
$_L$1129 Sal-BamHI f.	–	–	7.5µl	–
$_H$1129 EcoRI-BamHI f.	–	–	–	7.5µl
E	12µl	12µl	4.5µl	4.5µl
igase Buffer	4µl	4µl	4µl	4µl
igase	1µl	1µl	1µl	1µl

ncubate O/N at 16°C

-25-91

Transformed ligation mixtures into DH5α and plated on ~~50~~ LBAmp/X-gal

Results:

ligation	# colonies
pUC18 Sal-Bam	6 blue / 2 white
V_L1129	3 blue / 400 white
pUC 18 Eco-Bam	3blue / 1 white
V_H 1129	7blue / 88 white

picked 6 V_L1129 colonies and 6 V_H1129 colonies and grew in LBAmp (O/N in refrig.
(1-6) (7-12)

1-29-91

←pUC18
←hopefully supercoiled
← V_H1129

*will digest with less DNA or for longer time in order to see if soupercoiled ~~pat~~ band disappears

*will load more DNA of pUC18 V_L1129 to see if clones 1,2,3,4,6 have insert.

To Page No.____

Witnessed & Understood by me.	Date	Invented by KJ Bennett	Date 1/29/91
		Recorded by	

Project No._____

TITLE_____ ok No._____

From Page No.__ 1-30-91

Miniprep plasmid DNA sequencing:

- After 1ml ethanol option, dry pellet, resuspend in 100µl TE plus 100µg/ml RNase A
- Incubated @ 37°C for 30'
- phenol/chloroform extracted
- Added 35µl 20% PEG, 2.5M NaCl
- 30' on ice
- Spin 5'
- wash w/ 70% ethanol, vac dry 2X
- resuspended in 25µl TE

Use 2µg DNA template in 8µl volume.
Add 2µl 2M NaOH
Incubate at RT for 5 min
Add 3µl 3M NaOAc pH 4.5 (9 / 6 / 5)
2µl H_2O
5µl primer at 4 pmoles/µl (~~10µg/µl~~) : 5µl Sequencing Primer (Universal) (0.5pmo (GTAAAACGACGGCCAGT)
· 5µl SJ12 (reverse primer) 10µg/µl

75µl 100% ethanol for 5min, rinse w/ 70% ethanol 2X, vac dry, resuspend in 25µl TE

Annealing
2µg DNA 8µl of DNA/primer mix
2µl Sequencing buffer
Anneal by heating 2min at 65°C, then cooled slowly to <35°C

While cooling fill and cap tubes w/ 2.5µl of ea. termination mixture
Dilute labeling mix 1:5 to working conc. if needed. 8µl : 32µl H_2O
Dilute Sequenase for all templates in ice cold TE buffer 1:8 5µl : 35µl Exz Dil.
Pre-warm 4 termination tubes in 37°C bath

LABELING RXN: To 8µl annealed DNA mixture (10µl)
0.1M DTT 1µl
Diluted labeling Mix 2µl
[^{35}S]dATP 0.5µl
Diluted Sequenase 2µl
Incubate at RT, 5 min

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TERMINATION RXN

Transfer 3.5 µl of labeling rxn to each termination tube, mix and continue incubation of the term rxns at 37°C for 5 min

Stop rxn by adding 4 µl Stop Soln
Heat samples to 76°C for 2 min prior to loading

Gel mix: 42 g urea
5.8 g acrylamide
0.2 g bis
0.1 g APS
10 ml 10X TBS
100 ml total volume

oading order:

V_L 1129 #1 f.	V_H 1129 7f	−76-9r	#5
V_L 1129 2f.	V_H 1129 10f	−76-10r	#6
V_L 1129 5f.	V_H 1129 7r	−76-11r	
V_L 1129 1r.	V_H 1129 10r	−76-12r	
V_L 1129 2r. ✓	V_H 1129 12r		
V_L 1129 5r.			

itnessed & Understood by me,	Date	Invented by LJBennett	Date 1/30/91
		Recorded by	

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From Page No. 2 — 2-8-90

2

6% Sequencing gel: # 76-9 — V_H1129 # 7 r
76-10 — 8 r
76-11 — 9 r
76-12 — 10 r
V_H1129 # 7 f — 11 r
V_H1129 # 8 f — 12 r
10 f
11 f
12 f.

Primers: forward primer — 40 primer supplied by sequenase kit
reverse primer SJ12

Result: all V_H1129 clones sequenced are the incorrect heavy chain MOPC.
all V_L 1129 clones sequenced appear to be the correct sequence

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		Recorded by	2/8/91

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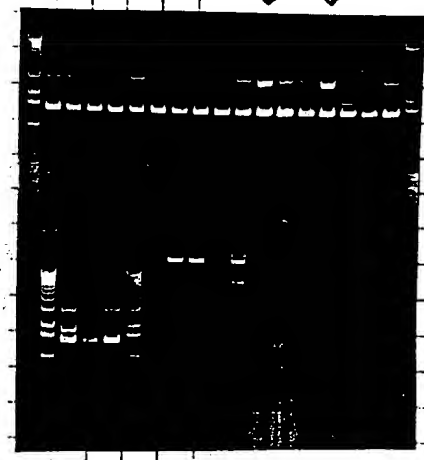
om Page No.__

2-11-91

- Picked 20 pUC 18 V_H 1129 clones from original transformation
- Analyzed each plasmid by digestion w/ Xba I
  - V_H 1129 contains Xba I site
  - MOPC21 does not contain Xba I site



1-20 : pUC 18 V_H 1129 Xba I
#10 uncut
#20 uncut

#11 and #14 appear to be the only correct clones
→ sequence 11, 14; (12 as negative)

Grow #11 and #14 up as midi prep.

3 Sequencing rxn:

1- pUC 18 V_H 1129 #11 (SJ12) reverse primer ←
2- pUC 18 V_H 1129 #12 (SJ12)
3- " #14 (SJ12)
4- pUC 18 V_H 1129 #11 (SJ12) ←
5- " #12 "
6- " #14 "
7-

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Witnessed & Understood by me.	Date	Invented by Z. Hashmeet	Date 2/11/91
		Recorded by	

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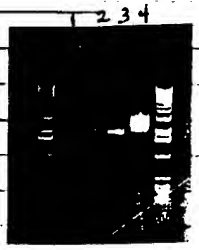
From Page No.__ 2/18/91

- Grew 2 ml cultures of 75+76 #1-3 to ~mid-log
- Infected with F1Ø by adding 10µl to 2 ml cultures
- Grew for 5 hrs.
- Spun down and saved supernatant in refrigerator

2-19-91

- Precipitated phage by adding equal volume 13% PEG 1.5M NaCl
- Ice for 30min
- Spun down for 15min at 10,000rpm
- resuspended in 300µl TE
- phenol/chloroform ext., chloroform ext., ethanol ppt, ethanol wash, c
- resuspended in 20µl TE



0.2 x 10 = 2µg

1-3) 75+76 (1-3)
4) SS DNA control from sequenase kit

1- V_H1129 #1A BamHI-EcoRI
2- V_H1129 #05 BamHI-SalI
3- V_H1129 #011 Bam-~~Sal~~ EcoRI

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Witnessed & Understood by me.	Date	Invented by JEBennett	Date 2/19/91
		Recorded by	

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From Page No.__ 2/20/91

4 Prep for sequencing V_H 1129 #11 & #14, V_L 1129 #5 midis; 75 + 76 #1, #2, #3:

V_H 1129 #11 } RNased, phenol/chloroform extraction
V_H 1129 #14 } PEG ppted 20% PEG, 2.5M NaCl
V_L 1129 #5 } ethanol washed 2x, dried
resuspended in 20μl TE

removed 8μl DNA
added 2μl 2M NaOH, incubated at RT for 5min.
added 3μl NaOAc pH 5.2, 2μl H_2O, 5μl primer at 4pmol/μl (10μg/μl) (SJ12)
added 75μl ethanol, spin 5min, wash with 70% ethanol, dry
resuspend in 8μl TE

Sequencing gel order

1 75+76 #1 r
2 75+76 #2 r
3 75+76 #3 r
4 V_L 1129 r
5 V_H 1129 r 11
6 V_H 11-9 r 14

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Witnessed & Understood by me.	Date	Invented by LLBennett	Date 2/20/91
		Recorded by	

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From Page No. 2/22

[redacted]

pSH1 HinDIII-Pst 36µl DNA
4µl React 2
2µl HinDIII
0µl PstI
1.5 hr incubation
gel fractionate
Gene Clean
resuspend in 20µl TE

Kinase treatment: pSH1 Pst-HinDIII 12µl
2µl 10X glycine-kinase buffer 2µl
2µl 10mM ATP 2µl
0.1M DTT 1µl
T4 Kinase 1µl
10µl

incubate at 37°C > 1hr. (too long)

Annealed SJ101µl (1µl) + SJ102 (µl) + 2µl Rxn Buffer + 6µl H_2O

65°C 3min, slow cooled.

Ligation reaction: 10µl pSH1 Pst-HinDIII (kinased)
10µl annealed linker
4µl Ligase Buffer
1.5µl Ligase

O/N incubation

RESULT: pSJ is the wrong vector to be using for this experiment. Repeat w pS18.

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Witnessed & Understood by me.	Date	Invented by L. Bennett	Date 2/22/91
		Recorded by	

m Page No.__

2/21

CR Reaction

Tube	Template	Primers	Fragment	Program
3	pUC18 V_L 1129 #5 midi 1 µl	SJ103 (1 mg/ml) 1 µl SJ104 (1 mg/ml) 1 µl	EcoRV-HindIII V_L 1129	2 min 94°C 1 min 94°C 2 min 60°C 2 min 72°C } 30X 8°C soak o/N

22

1	pUC18 V_L 1129 1 µl	~~SJ0~~ SJ103 (25 pmol/µl) 1 µl SJ104 "	V_L 1129 EcoRV-HindIII	2 min 94°C 1 min 94°C 2 min 48°C 2 min 72°C } 30X
2	pUC18 V_H 1129 1 µl	SJ105 SJ106	V_H 1129 f.	8°C soak o/N
3	cDNA 1129 1 µl	SJ105 SJ106	V_H 1129 f.	

ne by Sid 2/27/91

	Template	Primers	Fragment	Program
1	cDNA 1129	SJ105 SJ106	V_H 1129	1 min 94°C 2 min 37°C 2 min 72°C
2	pUC18 V_L 1129	SJ105 SJ106	V_H 1129	

Note: Used more Taq polymerase

Witnessed & Understood by me.	Date	Invented by	Date
		LJ Bennett	2/22/91
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From Page No. 2/22/91

SJ103 - 1129 and general VL 5' EcoRI, ClaI, EcoRV

EcoRI ClaI EcoRV

gCg gAA TTC AgA TCg ATA TCC AAC TgA CCC AgT CTC CA

STOCK CONC 1.0 mg/ml

(330)(38) = 12,540

$(12{,}540)^{-1}(1 \times 10^{-3}\ g/ml) = 8.0 \times 10^{-8}\ mol/ml = 80\ pmol/\lambda$

80x = (25)(100)

x = 31.25 µl in 100µl H_2O

SJ104 - 1129 VL 3' HindIII

TAT TTC AAg CTT ggT CCC CTA gCC gAA CgT

(330)(30) = 9900

$(9900)^{-1}(1 \times 10^{-3}) = 1.0 \times 10^{-7} = 100\ pmol/\lambda$

100x = (25)(100)

x = 25µl in 100µl H_2O

SJ105 - 1129 VH 5'

ggT TCT ggT AAA TCT TCC gAA ggT ggT AAA ggT CAg gTg CAg CTg CAg CAg TC(A,T) gg

(330)(56) = 18,480

$(18480)^{-1}(1 \times 10^{-3}) = 5.4 \times 10^{-7} = 54\ pmol/\lambda$

54x = (25)(100)

x = 46.3µl per 100µl H_2O

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Witnessed & Understood by me.	Date	Invented by L F Bennett	Date 2/22/91
		Recorded by	

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SJ106 - 1129, 98-6 VH 3'

~~BamHI~~ BamHI
gCg Agg ATC CTT ATg AgC TCA Cgg TgA C

$(330)(28) = 9240$
$(9240)^{-1}(1\times10^{-3}) = 1.1\times10^{-7} = 110\ pmol/\lambda$
$(110)X = (25)(100)$
$X = 23\mu l$ per $100\mu l$ H_2O

SJ108 - H1308 3' linker

HinDIII
Cgg AAg ATT TAC CAg AAC CAg Agg Tgg ACC CTT TTA TTT CAA gCT
Tgg TCC CC

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Witnessed & Understood by me.	Date	Invented by L Bennett	Date 2/22/91
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_____ Lab No. _____

From Page No. 2/23/91

Western blot to test J212-MB-KLH rabbit #1 antibody:

1-	Low MW Marker std.	4-20% gradient gel
2-	100ng 4-4-20/212	transferred to nitrocellulose 1 hr
3-	500ng 4-4-20/212	cut into three segments
4-	Low MW Marker std.	blott blocked 30min with milk
5-	100ng 4-4-20/212	blotted with 1°Ab at 1/200, 1/500, 1/1250 dilution
6-	500ng 4-4-20/212	washed
7-	Low MW Marker	blotted with 2°Ab at 1/3000 1 hr
8-	100ng 4-4-20/212	washed
9-	500ng 4-4-20/212	developed with ECL
10-		

1° Ab = J212-MB-KLH (rabbit) (test bleed)

2° Ab = goat-anti-rabbit 1/3000 IgG HRP

See blot notebook for results: Antibody reacts w protein at 1/1250 dilution

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Witnessed & Understood by me,	Date	Invented by	Date
		KJ Barnett	2/23/91
		Recorded by	

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From Page No. 2/28/91

WESTERN BLOT to determine titer of J212-MB-KLH
4-20% SDS-PAGE

1- Low MW	1- Low MW
2- 50ng 4-4-20/212	2- 50ng 4-4-20/212
3- Low MW	3- Low MW
4- 50ng 4-4-20/212	4- 50ng
5- Low MW	5- Low MW
6- 50ng 4-4-20/212	6- 50ng
7- Low MW	7- Biorad MW Std.
8- 50ng 4-4-20/212	8- G-98
9- Low MW Std.	9- G-126
10 50ng 4-4-20/212	10-

Transferred to nitrocellulose LR at 350m Amp
Blocked in 5% Milk O/N at RT
Blotted with J212-MB-KLH #1 & #2 Test bleeds at 1/1000, 1/5000, 1/12,500 } 4hr at RT
Blotted one strip with pre-bleed sera from rabbit #1 at 1/1000

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Witnessed & Understood by me.	Date	Invented by L Bennett	Date 2/28/91
		Recorded by	

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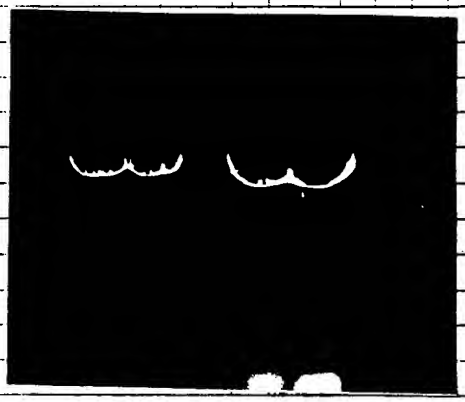
From Page No. 3/1/91

Digested pSH18 w/ HindIII + PstI : 36µl pS18
4µl React 2
2µl HindIII
2µl PstI

Digested pSH18 w/ HindIII : 5µl pS18
5µl H_2O
1.3µl react 2
1.0µl HindIII

Digested pS18 w/ PstI : 5µl pS18
5µl H_2O
1.3µl React
1.0µl Pst I

1- pS18 HindIII
2- pS18 PstI
3- pS18 PstI + HindIII
4- pS18 PstI + HindIII



Isolated the 3-4 fragments and gene Cleaned - resuspended in 40µl

To Page

Witnessed & Understood by me.	Date	Invented by A.J. Blewitt	Date 3/1/91
		Recorded by	

for the generation of $V_L + V_H$ 1129 by overlapping PCR:

V_L 1129 PCR fragment (from pg. 14): phenol/chloroform
chloroform
ethanol ppt
gel purify & Gene Clean

V_H 1129 PCR fragment: Sid cleaned
gel purified & Gene Cleaned

Preparative gel
1- V_L 1129
2- V_H 1129

[redacted]

PCR Reaction (overlapping PCR)

Tube	Fragment	Template	primers	Program
1	$V_L - V_H$ 1129	$V_L f + V_H f$ 1129	SJ103	94°C 2min
			SJ106	94°C 1min
				55°C 2min } 30X
				72°C 2min
				8°C soak

RESULT: NO AMPLIFICATION

$V_L - V_H$ 1129	$V_L f + V_H f$ 1129	SJ103	94°C 2min
	3µL 3µL	SJ106	94°C 1min
			45°C 2min } 10X
again, nothing			72°C 2.5min
			94°C 1min
			55°C 2min } 20X
			72°C 2.5min

Witnessed & Understood by me.	Date	Invented by	Date
		SJ Silmett	3/4/91
		Recorded by	

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From Page No. 19 3/4

Kinase treatment of pS18 PstI-HindIII fragment:

15µl pS18 PstI-HindIII f.
2µl 10X Ligase buffer
2µl 10mM ATP
1µl T4 Kinase
20µl total volume
45min at 37°C
heat inactivated at 65°C 10min

Annealed SJ101 and SJ102:
1µl SJ101 (1mg/ml)
1µl SJ102 (1mg/µl)
5µl H_2O
heated to 65°C 2min
slow cooled to < 37°C

Mixed 10µl pS18 + 3µl annealed SJ101-SJ102 + 1µl Ligase
incubated at 16°C for 2 hrs.

Transformed into HB101 (why not DH5-α?)
Result: 1 white transformant
picked white colony and grew as mini prep.

To Page

Witnessed & Understood by me.	Date	Invented by RJ Bennett	Date 3/4/91
		Recorded by	

[redacted]

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From Page No. 3/4/91

PCR to generate $V_L - V_H$ 1129

Tube	Fragment	Primers	Template	Program
				2min 94°C
1	~~pUC18 V_L 1129~~	SJ103	pUC18 V_L 1129	1min 94°C }
	V_L 1129	SJ104		2min 48°C } 30X
				2min 72°C }
2	V_H 1129	SJ105	pUC18 V_H 1129	2min 94°C 1min 94°C 2min 42°C } 10X 1min 94 2min 50 2min 72 2min 72°C 8°C soak
		SJ106		
3	$V_L - V_H$ 1129	SJ103	V_L 1129 f.	"
		SJ106	V_H 1129 f.	

V_H 1129 V_L 1129

[redacted]

Overlapping PCR did not work because the overlap is too small. Need to do another PCR reaction to extend the overlap on V_L 1129

3/5/91

Tube	Fragment	Primers	Template	Program
1	V_L 1129	SJ108 (1µl 1mg/ml)	V_L 1129 PCR 1µl	2min 94°C
	[redacted]	SJ103 (1µl 25pmol/µl)	(from above)	1min 94°C }
				2min 50°C } 30X
				2min 72°C }
				8°C soak

Note: V_L 1129 PCR 1 not cleaned at all; used straight from PCR reaction.

To Page

Witnessed & Understood by me.	Date	Invented by Lisa J Bennett	Date 3/5/91
		Recorded by	

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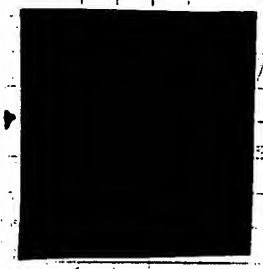
Overlapping PCR to generate V_L 1129 – V_H 1129

Tube	Fragment	Template	Primers	Program
4(1)	V_L–V_H 1129	V_L 1129 103-108	SJ1103	94°C 2min
		purified V_H 1129 105-104	SJ1106	94°C 1min 45°C 2min } 30X 72°C 2min 8°C soak

← expecting a ~750 bp fragment

3/7	Fragment	Template	Primers	Program
2	V_L–V_H 1129	V_L 1129 103-108 (1 µl)	SJ1103	
		purified V_H 1129 105-106 (1 µl)	SJ1106	94°C 2min
				94°C 1min }
3	V_L–V_H 1129	SJ103-SJ106 (above PCR)	SJ103	55°C 2min } 30X
			SJ106	72°C 2min
				8°C soak



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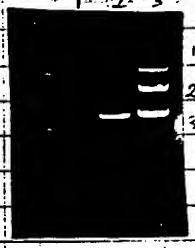
tnessed & Understood by me.	Date	Invented by Silas Bennett	Date 3/7/91
		Recorded by	

Project No.

TITLE Book No.

From Page No.

3/6/91

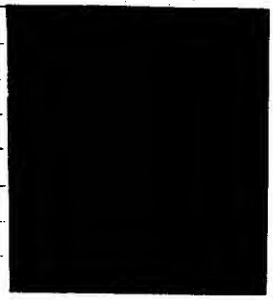


1- pCMVPA 5µl
2- 2627 5µl (Sid vector)
3- ~~pCMV~~ 5410 5µl (Sid vector)

Digested 10µl 5410	14µl 2627	20µl pCMVPA
26µl H_2O	22µl H_2O	16µl H_2O
4µl React 3	4µl React 1	4µl React 1
2µl BamHI	1µl ClaI	1µl ClaI
BglI stupid 2µl BglII	1hr digestion	1hr digestion
1hr digestion	add 0.4µl 3M NaCl	add 0.4µl 3M NaCl
BglI! →	2µl BglII	2µl BamHI
	incubate 1hr.	incubate 1hr.

Fractionate on 1% preparative gel
Isolate fragments and clean w Gene Clean

3/7/91



Redigestion of 5410 and 2627 w BglI this time:

10µl 5410	14µl 2627
26µl H_2O	22µl H_2O
4µl React 2	4µl React 2
2µl BamHI	2µl BglI
2µl BglI	2µl ClaI
1hr incubation at 37°C	1hr incubation at 37°C

Prep.
Gel 1- 5410 BamHI-BglI
2- 2627 ClaI-BglI
Isolated pCMVPA 1000 Kb ClaI-BamHI f.
Isolated 3000 Kb 5410 BamHI-BglI f.
Isolated 1000 Kb 2627 ClaI-BglI f. } Gene Cleaned

To Page

Witnessed & Understood by me.	Date	Invented by BJBennett	Date 3/7/91
		Recorded by	

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rom Page No. 25

Resuspended ea. fragment in 5µl TE.
Mixed fragments for Ligation: 15µl DNA
2µl Ligase Buffer (10X)
2µl 10mM ATP
1µl Ligase
Incubated O/N at 16°C

'8/91
Transformed into DH5-α and plated on LBAmp media.

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Witnessed & Understood by me.	Date	Invented by Lisa S. Bennett	Date 3/8/91
		Recorded by	

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Book No.

From Page No. 3/16/91

SDS-PAGE GEL (for Sid) 4-20% Gradient Gel

— no apparent expression on this gel Western Sid has the blots —

1- 4-4:20 std. 50~~ng~~ µl

2- super. #1

3- #2

4- #3

5- #4

6- LMW Std

7- pellet #1

8- #2

9- #3

10- #4

Transferred to nitrocellulose

Blocked w 5% milk for 30 min

Blotted w 1° Ab (rabbit J212-MB-KLH #1) 1/2000 for 30 min

Washed

Blotted w 2° Ab (goat anti-rabbit IgG-HRP) 1/3000 for 30 min

Washed

Developed with ECL detection System

To Page

Witnessed & Understood by me:	Date	Invented by SCHMIDT	Date 3/6/91
		Recorded by	

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rom Page No. 23

3-8-91

- Cleaned PCR fragment : phenol/chloroform ext., chloroform ext., ethanol ppt., ethanol w
- Digested w/ EcoRI-BamHI : 36µl V_L-V_H 1129 Aref. (resuspended in TE)
  4µl React 3
  2µl EcoRI
  2µl BamHI
  Incubate 1 hr at 37°C
- Digested pUC18 w/ EcoRI + BamHI : 10µl pUC18
  26µl TE
  4µl React 3
  2µl EcoRI
  2µl BamHI
  Incubate 1 hr. at 37°C.

Preparative Gel to Isolate V_L-V_H 1129 750bp band & pUC18 Eco-Bam
Eco-BamHI

1 2

[redacted]

1-V_L-V_H 1129 EcoRI-BamHI
2 pUC18 EcoRI-BamHI

Isolated these fragments, Gene Cleaned
resuspended V_L-V_H 1129 in 10µl
pUC18 Eco-BamHI in 20µl

Ligation Reaction

10µl V_L-V_H 1129 EcoRI-BamHI
5µl pUC18 EcoRI-BamHI
2µl 10X Ligase Buf.
2µl 10mM dATP
1µl ligase
incubated ~~16°C~~ 16°C for 45 min

Transformed into DH5-α, outgrow 1 hr, plate on LBAmp

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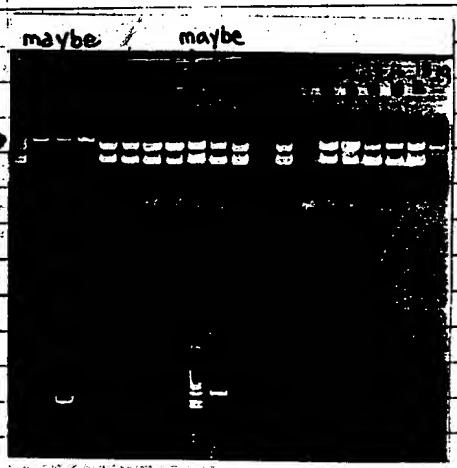
Witnessed & Understood by me.	Date	Invented by	Date
		J. Bennett	3/8/91
		Recorded by	

From Page No. 24 3-11-91

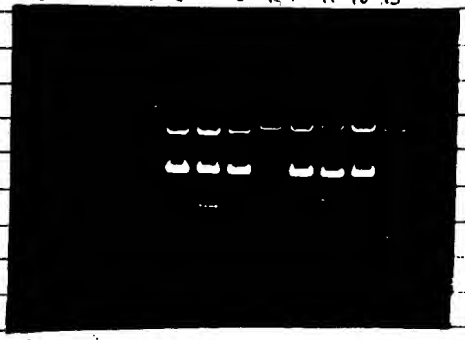
• Started miniprep to screen 5410-2627-pCMVPA (1-3)
pUC18 V_L-V_H (4-18)
pUC18 (19-20)
} 2ml in LBAmp

screen 5410-2627-pCMVPA (1-3) w/ EcoRI → to linear form ~ 5000 Kb
screen pUC18 V_L-V_H (4-18) w/ EcoRI
pUC18 (19-20) w/ EcoRI
1-15, 17, 18, 18-20



1-3) appear to be the correct size (5000
need to cut with enzyme from the lu
HinDIII, Sma or Sal

4-18) pUC18 V_L-V_H transformation
#5, #8, #9 may contain insert
2.7Kb pUC18 band appears to be sl
larger.
Additional screening: EcoRI + B
(to cut out insert)
Also digest w/ HinDIII



1- #1 BglI-SalI ✓
2- #2 BglI-SalI ✓
3- #3 BglI-SalI ✓
4- 5410 BglI-SalI
5- 2627 BglI-SalI ✓
6- #5 EcoRI-BamHI ✓
7- #8 EcoRI-BamHI ✓
8- #9 EcoRI-BamHI -
9- #20 EcoRI-BamHI ✓
10- #5 HinDIII ✓

Grow #1, 2, 3, 5, 8, 9 midipreps. (all in L

NOTE: Oligos 5110 - 5118 were received today, 3-11-91.

11- #9 HinDIII
12- #8 HinDIII ✓
13- #20 HinDIII ✓

To Pag

Witnessed & Understood by me.	Date	Invented by	Date
		Recorded by	3/11/91

From Page No. 3-11-91

PCR to generate more V_H1129 105-106:

Tube	Fragment	Template	Primers	Program
11	V_H1129 105-106	pUC18 V_H1129 #11	SJ105 (fresh from stock 25 pmol/μl	94°C 2min
14		#14	SJ106	94°C 1min
				46°C 2min } 10X
				72°C 2.5min
				94°C 1min
				55°C 2min } 20X
				72°C 2min
				8°C soak

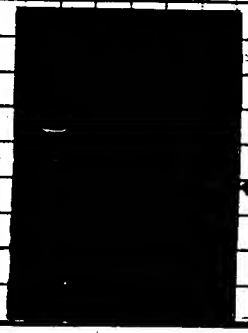
← SJ105-SJ106

Preparative gel 11414

← Isolated this 400 bp band to GeneClean

3-12-91

Template Fragment	Fragment	Primers	Program
V_L 1129 103-108 + V_H1129 105-106	V_L+V_H 1129	SJ103 (fresh 5 pmol/μl)	94°C 2min
		SJ106 (yesterday)	94°C 1min
			42°C 2min } 10X
			72°C 2min
			94°C 1min
			55°C 2min } 20X
			72°C 2min
			8°C soak.



← Isolate this fragment

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		Recorded by	

TITLE _____ Project No. _____

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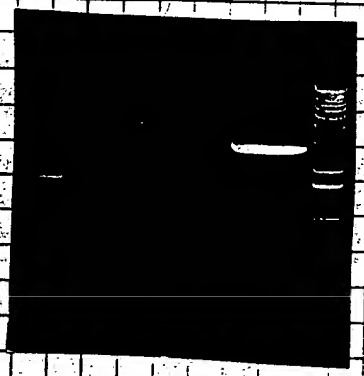
From Page No. 29

- Pooled 5410-2627-pCMVPA clones 1, 2, 3 (minipreps)
  Brought volume up to 36µl.
  Digested with HinDIII and SalI

- Brought volume of pUC18 #2 miniprep to 36µl.
  Digested with HinDIII - EcoRI



1- 5410-2627 pCMVPA Cla + BamHI #1
2- #2
3- #3
4- 2627 Cla - BamHI
5- 5410-2627 pCMVPA BglI + BamHI #1
6- #2
7- #3
8- 2627 BglI - BamHI
9- 5410-2627 pCMVPA Cla + BglI #1
10- #2
11- #3
12- 2627 Cla + BglI
13- pUC18 V_L-V_H 1129 #5 EcoRI + BamHI
14- #8 "
15- #9 "
16- pUC18 EcoRI + BamHI
17- pUC18 V_L-V_H 1129 #5 HinDIII
18- #8 HinDIII
19- #9 HinDIII
20 pUC18 #20 HinDIII

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Witnessed & Understood by me, _____ Date _____

Invented by Linda Bennett Date 3/11/91

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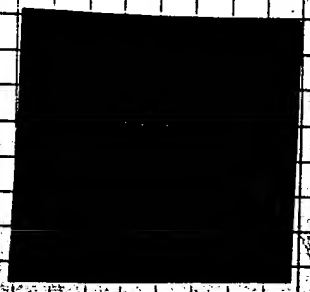
From Page No. 31 3-15-91

Construction of chimeric expression vector

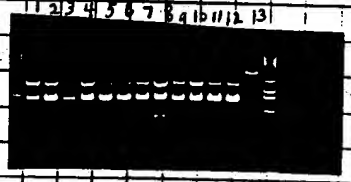
36µl 2627	18µl 5401	5µl pCMVPA
4µl React 2	3µl React 2	13µl H_2O
2µl ClaI	1µl BamHI	2µl React 1
2µl BglI	1µl BglI	1µl ClaI
		1µl BamHI

1- 2627 ClaI-BglI
2- 5401 Bam-BglI
3- pCMVPA Cla-BamHI



Isolated the fragments (circled) and GeneCleaned in one tube
Resuspended in 1x ligase buffer (18µl)
Ligated in 20µl total volume at 4°C
Sid transformed into E. coli

Result —



1-11?) 5401-2627 pCMV Bam-EcoRI ← wrong, looks like pUC
13) 2627 Bam-EcoRI

To Page No. _____

Witnessed & Understood by me,	Date	Invented by DJ Schmidt	Date 3/15/91
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[redacted]

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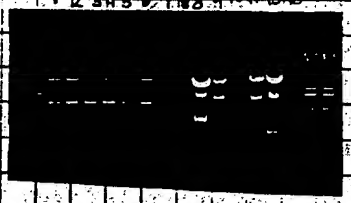
From Page No.___

5µl pUC18 digested w/ EcoRI and HinDIII
fragment purified and GeneClesned.
annealed ~~primers~~ oligos S1117 and S1118 1µl of ea in 20µl volume.
slow cooled.
ligation mixture: 5µl pUC18 EcoRI + HinDIII
1µl S1117 + S1118
2µl 10X ligase buf
2µl dATP
10µl H_2O
1µl Ligase

Result: ~250–300 colonies (100µl of cells)
~2–4 blue colonies

Transformed into DH5-α; plated on LBAmp + X-gal.

3-20-91



3-21-91
- started 50ml culture of pUC18 Nco-Not #1 & #2
- sequencing both of these, also see pg. 37

1- pUC18 link #1 NcoI
2- " #2 NcoI
3- " #3 NcoI
4- " #4 NcoI
5- " #5 NcoI
6- " #6 NcoI
7- pUC18 NcoI
8- pUC18 V_L-V_H 1129 #5 EcoRI-BamHI
9- " #9 EcoRI-BamHI
10- pUC18 EcoRI-BamHI
11- pUC18 V_L-V_H 1129 #5 HinDIII
12- " #9 HinDIII
13- pUC18 HinDIII

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Witnessed & Understood by me,	Date	Invented by L. Blumett	Date 3/20/91
		Recorded by	

Project No. [illegible]

TITLE _____ Book No. [illegible]

From Page No. 3-2-91

Sequencing of pUC18 V_L-V_H #5 & #9, p5401-2627-cmv #4, pUC18, pUC18 Nco-Not 1,3, 75+7

5

Followed procedure on pg 5:

Reactions:

1- puc 18 V_L-V_H #5 midi forward primer SJ13 ✓
2- puc 18 V_L-V_H #5 midi SJ112 ✓
3- #5 midi SJ12 ; reverse primer ✓
4- pUC18 V_L-V_H #9 midi SJ13 ✓
5- pUC18 V_L-V_H #9 midi SJ112 ✓
6- pUC18 V_L-V_H #9 midi SJ12 ✓
7- p5401-2627 pCMV #4 mini SJ13
8- pUC18 midi ~~SJ~~-40
9- pUC18 Nco-Not #1 mini -40
10- pUC18 Nco-Not #3 mini -40
11- 75+76 #1 mini SJ12 } not correct
12- 75+76 #2 mini SJ12 } do not contain the desired mutati

PEG ppted (13% PEG, 1.5M NaCl) pUC18 V_L-V_H #9
p5401-2627-pCMV
pUC18
pUC18 Nco-Not 1 & 3
75+76 1,2,3

		A_{260}	dilution		extinction coeff	
conc.	pUC18 V_L-V_H #5:	0.51	x 200	= 102 x 50	=	5100 µg/ml = 5.1 µg/µl
	pUC18 V_L-V_H #9:	0.263	x 200	= 52.6 x 50	=	2630 µg/ml = 2.63 µg/µl
	pUC18 :	0.47	x 200	= 94 x 50	=	4700 µg/ml = 4.7 µg/µl

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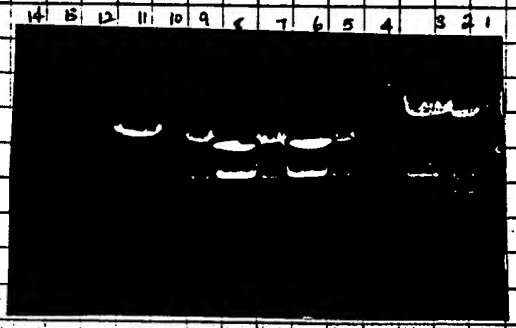
Witnessed & Understood by me,	Date	Invented by D Bennett	Date 3/21/91
		Recorded by	

TITLE _____ Project No. _____ Book No. _____

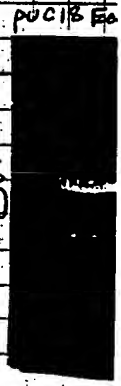
From Page No. 3/25/91

1- MW Marker
2- pS134 HinDIII
3- pST34 HinDIII + NcoI
4- pS132 NcoI + SacI
5- pUC18 link HinDIII
6- pUC18 link Nco + HinDIII
7- pUC18 link Nco
8- pUC18 link Nco + SacI
9- pUC18 SacI
10- pUC18 V_L1129 EcoRI
11- pUC18 V_L1129 EcoRI - HinDIII
12- pUC18 V_H1129 PvuII
13- pUC18 V_H1129 PvuII + SacI
14- MW Marker



↑ ↑ ↑ ↑ Isolated these fragments and gene Cleaned.

Transformed the following DNAs into DH5-α:
S132 : V_H1308 + signal in 2627 derived vector
S134 : V_L1308 + signal in 2627 derived vector
S136 : 5401-2627 + pCMVPA #1 (from Sid)
Plated on LBAmp
*Result: approximately 300 colonies/plate



Digest pUC18 link w EcoRI + HinDIII (for frag. isolation tomorrow)

Ligations:

	pUC18 32	pUC18 Nco-Sac	pUC18 34	pUC18 Nco-HinD
pUC18 Nco-Sac	1µl	1µl	—	—
S132 Nco-Sac	4µl	—	—	—
pUC18 Nco-HinD	—	—	1µl	1µl
S134 Nco-HinD	—	—	4µl	—
10X Lig. Buf.	2µl	2µl	2µl	2µl
10X ATM	2µl	2µl	2µl	2µl
	1µl	1µl	1µl	1µl
	15µl	15µl	15µl	15µl

To Pag

Witnessed & Understood by me, _____ Date _____

Invented by D Emmitt

Recorded by

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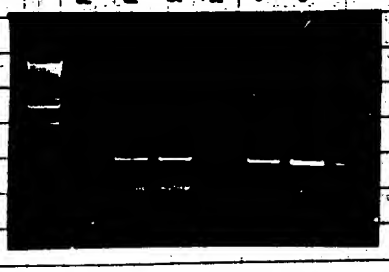
om Page No.__ 3/25/91

PCR Reaction

Tube	Fragment	Template	Primers	Program
A	~~98-6 cDNA~~ HuCkappa w 5' V_L1129 overlap	98-6 cDNA	SJ1114 SJ1116	94°C 2min 94°C 1min 50°C 2min 72°C 2min } 30X 8°C soak
B	V_L1129 w HuCkappa overlap	V_L1129 1103-104	SJ103 SJ115	

SJ114: (330)(40) = 13,200
$(13{,}200)^{-1}(1\times10^{-3}) = 7.57\times10^{-8} = 75.7$ pmol/λ
25(100) = x(75.7)
x = 33.02 µl

SJ116: (330)(30) = 9900
$(9900)^{-1}(1\times10^{-3}) = 100$ pmol/λ
25(100) = x(100)
x = 25 µl



SJ103: (330)(38) = 12,540
$(12{,}540)^{-1}(1\times10^{-3}) = 7.97\times10^{-8} = 79.7$ pmol/λ
25(100) = x(79.7)
x = 31.37 µl

~~• pooled mix~~

SJ115: (330)(40) = 13,200
75.7 pmol/λ
x = 33.02 ~~pmol~~ µl/100 µl

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Witnessed & Understood by me,	Date	Invented by	Date
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From Page No. 40 3/26/91

Overlapping PCR

Tube	Fragment	Template	Primers	Program
A (1-4)	mV_L 1129-huCK	mV_L 1129 103-105 (1µl)	SJ103 (25pmol/2)	94°C 2mi
		huCK 114-116 (1µl)	SJ116 (25pmol/2)	94°C 1min
				50°C 2mi
				72°C 2mi
				~~8°C soak~~
				55°C 2mi
				72°C 2mi
				8°C soak

[redacted]

← isolate these fragments after digestion with EcoR1-HinDIII

- pooled mV_L 1129 - huCK PCR reactions; phenol/chloroform, chloroform, ethanol ppt, v
- digested w/ EcoR1 - HinDIII (for cloning into puc18)
- gel purified and GeneClean

[redacted]

← isolated both fragments

Note: Actually there is no HinDIII site in this fragment; the 5' and 3' ends should contain EcoR1 sites

To Page N

Witnessed & Understood by me,	Date	Invented by Susan J. Barnett	Date 3/26/91
		Recorded by	

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n Page No. 40

1/26/91 huCK-pPa 114-116 fragments
- pooled mV1129 103-115 fragments; cleaned, ethanol ppted, resuspended in 40µl TE.
- removed 20µl of ea., brought volume to 36µl
- digested both fragments w EcoRI-HinDIII for cloning into pUC18
- gel purified and gene Cleaned

Eco-HinD mV1129 | Eco-HinD huCK

[redacted]

Ligations:

	pUC18 mV1129 103-115	pUC18 huCK 114-116	pUC18 Eco-HinD
mV1129 EcoRI-HinDIII 103-115	4µl	–	–
huCK EcoRI-HinDIII 114-116	–	4µl	–
pUC18(linE) EcoRI-HinDIII	1µl	1µl	1µl
TE	10µl	10µl	14µl
Ligase Buf (10x)	2µl	2µl	2µl
10mM ATP	2µl	2µl	2µl
ligase	1µl	1µl	1µl

O/N at 4°C

7. Transformed ligations into DH5-α
Plated on LBAmp plates after 1 hr. outgrowth in S.O.C
+X-gal

8
Results

	# colonies
pUC18 Eco-HinD	~200 white
pUC18/103-115	~1000 colonies, white, ~~blue~~, light blue
pUC18/114-116	~1000 colonies, white, blue, dark blue

To Page No. ____

essed & Understood by me,	Date	Invented by Dina L. Bennett	Date 3/28/91
		Recorded by	

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From Page No. 39

3/26/91

Transformed ligated DNAs into DH5-α:
5µl pUC18 Nco-SacI (negative control)
5µl pUC18-SJ32 Nco-Sac
5µl pUC18 Nco-HinDIII
5µl pUC18-SJ34 Nco-HinDIII
plated on LBAmp plates after 1.5 hr outgrowth

Transformed SJ33 and SJ35 DNAs to make stock (see Sid's notebook for details and description of plasmids)
- into DH5-α
- 1µl SJ33 and SJ35
- plated on LBAmp after 1.5 hr outgrowth.

Transformed SJ36+•(117-118) (Sid's ligation reactions) into DH5α:
5µl of SJ36+(117-118) [+]
5µl SJ36 religation [-]
plated on LBAmp after 1.5 hr outgrowth.

Results:

pUC18 Nco-Sac	177	uniform
pUC18/32	600-1000	uniform colony size
pUC18 Nco-HinDIII	250	uniform
pUC18/34	~500-600	big and small colonies
SJ33	~1000	very small colonies
SJ35	~5000	uniform
SJ36+(117-118)	150	ligations did not appear to work
SJ36 −	150	

To Page

Witnessed & Understood by me,	Date	Invented by Dana J. Bamford	Date 3/27/91
		Recorded by	

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From Page No. 43

3/28/91

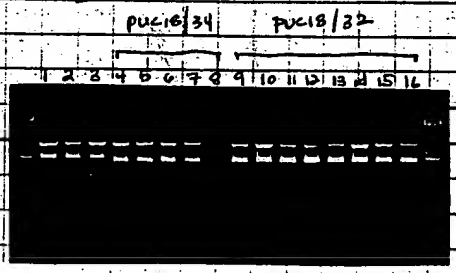
Picked colonies for minipreps and restriction digestion analysis:

		Results
SJ33	1-3	very slow growing
SJ35	4-6	
puc18/34	(7-10 big) & (11-14 small)	11,12,14 very slow growing
puc18/32	15-22	

grew in LBAmp O/N

pSJ33: hV1308F V_L Nco + HinDIII

pSJ35: hV1308FGA V_L-V_H Nco + BamHI



1- pSJ35 Nco-HinDIII #4
2- " #5
3- #6
4- puc18/34 Nco-HinDIII #7
5- #8
6- #9
7- #10
8- #13
9- puc18/32 Nco-SacI #14
10- #15
11- #16
12- #17
13- #18
14- #19
15- #20
16- #21
#22

pick #7 (puc18/34) } to grow for midi prep culture.
#15 (puc18/32) }

4/2 Completed midi prep for puc18/34 #7 (Nco-Not)
puc18(Nco-Not)/32
pSJ35

To Page No.

Witnessed & Understood by me,	Date	Invented by Lisa Bennett	Date 3/28/91
		Recorded by	

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From Page No. 41

Construction of puc 18 - mV1129 - huCK

Ligation Reactions:

	puc18 mV1129-huCK	puc18 EcoRI
puc 18 EcoRI (Inter)	1 µl	1 µl
mV1129-huCK EcoRI f	4 µl	—
TE	10 µl	14 µl
Ligase Buffer (10X)	2 µl	2 µl
10mm ATP	2 µl	2 µl
ligase	1 µl	1 µl

incubated at 4°C for 1.5 hr

puc18 (Nco-Not)	10 µl
TE	26 µl
React 3	4 µl
EcoRI (100U/µl)	2 µl

digested at 37°C 1.5 hr, phenol/chloroform, chloroform, ethanol ppt, wash

transformed ligated DNAs into DH5-α

plated on LBAmp + X-gal after outgrowth (1hr.) in SOC

308 Result:

puc18 mV1129-huCK	~~2000~~ colonies	no blue colonies however
puc18 EcoRI	~~2000~~ colonies	

Explanation: this vector is out of frame w/ lacZ so unless a gene which is in frame is inserted all colonies will be white

Note: ~~Better to store~~

To Page

Witnessed & Understood by: Date: Invented by: David J. Bennett Date:

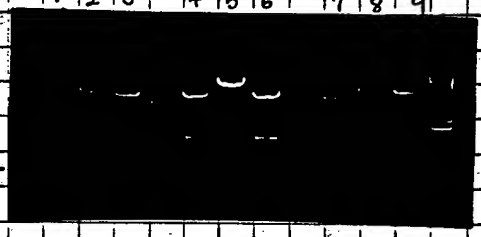
Recorded by:

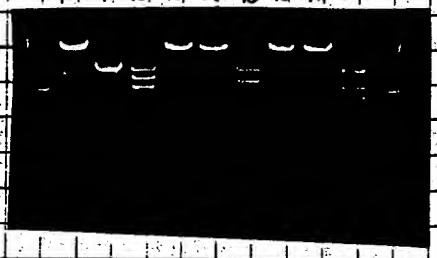
TITLE _____ Project No. _____ Book No. _____

From Page No. 3/27/91

Restriction enzyme digestion analysis of SJ32, SJ34, SJ36, pCMVpA.

1- SJ32 Nco+ Sac } V_H
2- SJ32 Nco
3- SJ32 Sac
4- SJ34 Nco-HinDIII } V_L
5- SJ34 Nco
6- SJ34 HinDIII
7- SJ36 EcoRI-BamHI
8- SJ36 HinDIII
9- SJ36 SalI
10- pCMVpA Cla
11- EcoRV
12- EcoRI
13- BglII
14- SalI
15- SmaI
16- HinDIII
17- BamHI
18- EcoRI-Bam





Preparative Gel to Isolate pSJ36 HinDIII-SalI fragment

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Witnessed & Understood by me,	Date	Invented by R Bennett	Date 3/27/91
		Recorded by	

rom Page No. 47

3/28/91 SJ36 + (NcoI-NotI linker) Construction

- Isolated SJ36 HinDIII-SalI fragment from prepariative gel
- Gene Cleaned
- Annealed 1μl SJ110 + 1μl SJ111 (1μg/2) w/ 1X Reaction Buf, ~~2μl~~ 14μl H_2O 2min 65°C, cooled slowly to room temp.
- Ligated pSJ36 HinDIII-Sal + (SJ110-SJ111)

		control
pSJ36 HinDIII-SalI	2μl	2μl
SJ110-SJ111	2μl	—
H_2O	11μl	13μl
10X Ligase Buffer	2μl	2μl
10mM ATP	2μl	2μl
Ligase	1μl	1μl

incubated at room temperature for 3 hr.

- Transformed into DH5-α and plated on LBAmp media
- Incubate at RT over-weekend

- Transformation did not work : probably because on of the enzymes did not cut all the way.

The DNA was cut w/ HinDIII + SalI in React 3
HinDIII doesn't cut well in React 2.

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		Recorded by	

Project No.

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From Page No. 42

- Picked colonies to grow minipreps and analyze by restriction digestion

4/1

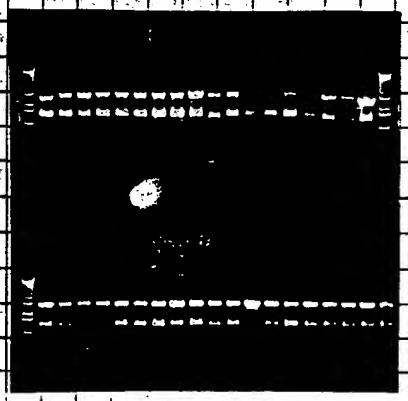
1-5) puc18/mV 1129 103-115 blue
6-10) puc18/mV 1129 103-115 white
11-15) puc18/mV huCK 114-116 blue
16-20) puc18/huCK 114-116 white
21-36) puc18/mV 1129 + huCK (white)

- Kept in refrigerator

4/2

Completed minipreps and analyzed by restriction digestion.

1-10) puc18(Nco-Not)/mV 1129 103-115 EcoRI + HindIII
11-20) puc18(Nco-Not)/huCK 114-116 EcoRI + HindIII
21-36) puc18(Nco-Not)/mV 1129-huCK EcoRI



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		L Bennett	4/2/91

rom Page No.__

4/1/91

INSERTION OF NcoI-NotI linker INTO pSJ36

• Digested pSJ36 with HinDIII + SalI

36µl pSJ36
4µl React 2
2µl HinDIII
incubated 1hr. at 37°C
added 0.6µl 3M NaCl
2µl SalI
incubated 1hr. at 37°C
heat killed
fractionated prep on 1% agarose gel
GeneCleaned

1- pSJ36 HinDIII-SalI
2- pUC18(Nco-Not) EcoRI-BamHI
3- pUC18 V_L-V_H 1129 EcoRI-BamHI

Ligation

	pSJ36 HinD-SalI SJ110-111	pSJ36 HinD-SalI
pSJ36 HinDIII-SalI	1µl	1µl
SJ110+SJ111	3µl	—
10X Ligase Buffer	2µl	2µl
10mM ATP	2µl	2µl
H_2O	11µl	14µl
Ligase	1µl	1µl

Incubated at 4°C O/N

4/2/91

• Transformed into DH5-α and plated on LBAmp

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4/1/91

verlapping PCR Reaction to generated mV$_L$129 - huCKappa chimeric

Template	Fragment	Primers	Program
mV$_L$129(93-115)	mV$_L$129-huCK	S\103 (100pmol/2)	
huCK(114-116)		S\116 (100pmol/2)	94°C 1 min 65°C 2 min 72°C 2 min 8°C soak } 30X
(purified frag.)			

a b c d

• Threw these reaction out because previous experiment (at 50°C) was better

4/2/91

• Diluted template DNA 1/10 and 1/100 and redid the exp at 50°C annealling tem

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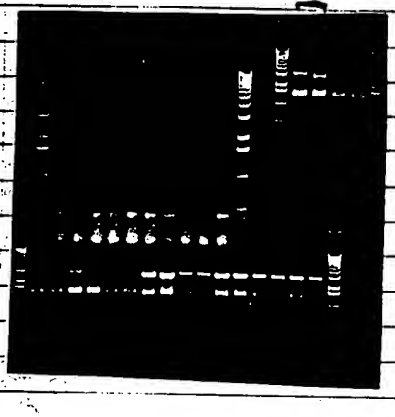
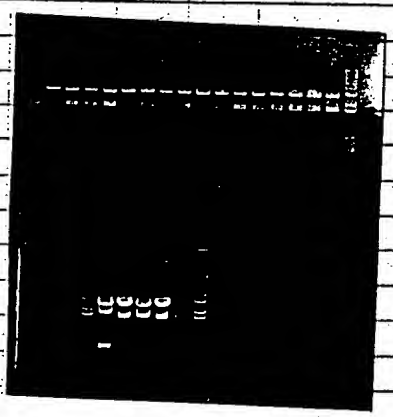
4/3/91

Restriction enzyme analysis of pUCN-N huCk and pUCN-N V_L-huCk

1- pUCN-N huCk EcoRI #11
2- EcoRI - HinDIII #11
3- #12
4- #12
5- #13
6- #13
7- #14
8- #14
9- #15
10- #15
11- #16
12- #16
13- #17
14- #17
15- #18
16- #18
17- #19
18- #19
19- #20
20- #20

21- pUCN-N V_L #29 - huCk EcoRI - HinDIII #21
22- #22
23- #23
24- #24
25- #25
26- #26
27- #27
28- #28
29- #29
30- #30
31- #31
32- #32
33- #33
34- #34
35- #35
36- #36
37- pSJ35 Nco - HinDIII ✓
38- pUCN-N Nco - HinDIII ✓
39- pUCN-N/34 Nco - HinDIII ✓
40- pUCN-N Nco - Sac ✓
41- pUCN-N/32 Nco - Sac ✓





none of the puc V_L-huCkappa 2p to be correct.

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Date

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3/8/91

Preparative Gel

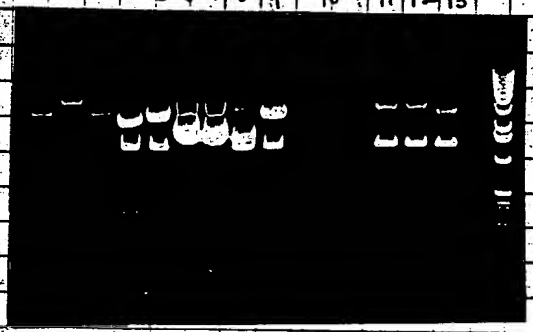
1- pUCN-N/32 PvuII-EcoNcoI
2- pUCN-N/34 EcoRV-NotI
3- V_L129-huCKappa EcoRI-NotI

4- pUCN-N/82 PvuII-NcoI
5- pUCN-N EcoRI-NotI

overloaded

1- pUCN-N/32 PvuII
2- NcoI
3- PvuII+NcoI
4- pUCN-N PvuII
5- pUCN-N NcoI
6- pUCN-N/34 EcoRV
7- NotI
8- pUCN-N EcoRV
9- pUCN-N NotI
10- V_L-huCKappa f. Eco-Not
11- pUCN-N V_L129 EcoRV ✓
12- HindIII ✓
13- EcoRV-HindIII ✓

1 2 3 4 5 6 7 8 9 10 11 12 13



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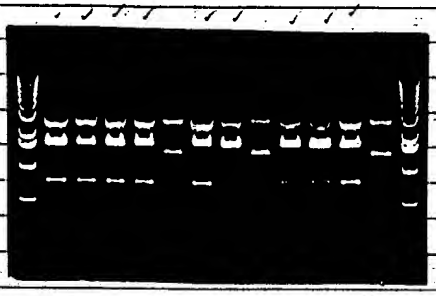
9/3/91

Minipreps and restriction enzyme analysis of pUC N-N V_L-V_H 1129 & pSJ36 (110-111)

1-12) pUC N-N V_L-V_H

13-24) pSJ36 (110-111)



1-12) pUC N-N V_L-V_H 1129 EcoRI-BamHI

13-24) pSJ36 (110-111) NcoI

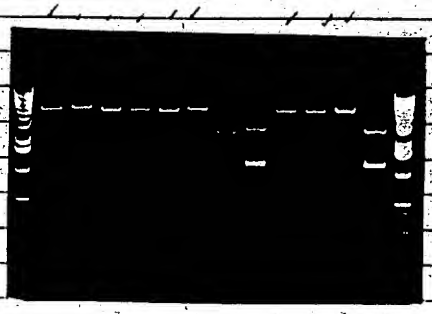
25- pUC N-N CK EcoRI (midi

26- HindEcoRI

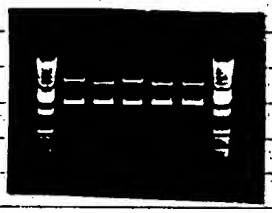
27- HinD

28- HinD+NotI

29- NotI's check this again



Grew midi cultures of #2, #3, 14, 15



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4/4/91

PCR Reactions

Tube	Fragment	Template	Primers	Program
A	V_L 1129 103-115	~~pUC19 1129~~ V_L 1129 103-104	SJ103 SJ115	94°C 2 min; 94°C 1 min, 52°C 2 min, 72°C 2 min } 30x
B	huCkappa 114-116	~~p 98 ...~~ pUC-huCK	SJ114 SJ116	8°C soak
C	V_L 1129-huCkappa	V_L 1129 103-115, huCK 114-116	SJ103 SJ116	
D	huCK 22-123	268 SO.128 mp	SJ22 ~~[illegible]~~ SJ123	
E	V_L 1129 103-115	V_L 1129 103-115	SJ103 SJ115	
F	huCkappa 114-116	huCkappa 114-116	SJ114 SJ116	

4/5/91

↓ ↓ ↓ ↓ ↓ Put reaction back in Perkin-Elmer machine for 10 more cy

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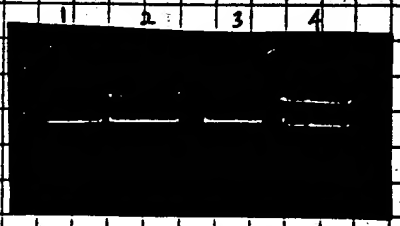
4/5/91

Construction of Signal - V_L 1129 - huCkappa in pUC18-N

A. Pooled pUC18-N/V_L 1129 103-115 mini DNAs #1,2,3
Brought volume to 36µl
Added 4µl React 2
3µl EcoRV
3µl HinDIII

B. Digested 36µl pUC18-N/huCkappa with HinDIII + NotI
4µl React 2
3µl HinDIII
3µl ~~HinDIII~~ NotI

C Pooled pUC18-N/34 mini DNAs # 7, 8, 9
Brought volume to 36µl
Added 4µl React 2
3µl EcoRV
3µl NotI



Preparative gel
1- pUC18-N huCkappa HinDIII-NotI
2- pUC18-N/34 EcoRV-NotI
3- pUC18-N V_L 103-115 EcoRV-HinDIII
4- pUC18-N Nco-SacI

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15/91

igations

	pUCN-N signal-V_L-huCk	pUCN-N signal-V_H
pUCN-N/V_H1129 EcoRV-HinDIII	5µl	–
pUCN-N/huCkappa HinDIII-Not	5µl	–
pUCN-N/34 EcoRV-NotI	5µl	–
pUCN-N/32 PvuII-NcoI	–	5µl
pUCN-N NcoI-SacI	–	3µl
V_H1129 PvuII-SacI pcr f.	–	5µl
10X ligase buffer	2µl	2µl
10mM ATP	2µl	2µl
Ligase	1µl	1µl
H_2O	–	3µl

Incubate at 4°C over weekend

1-8-91

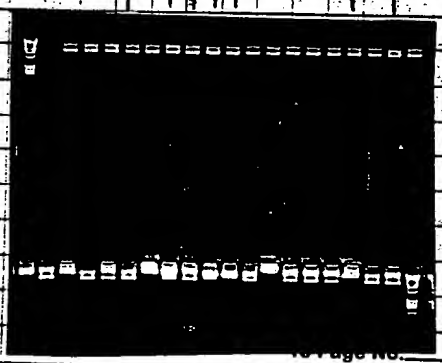
Transformed ligations into DH5-α ion LBAmp-Xgal plates
Also transformed pUCN-N/34 #10, pUCN-N/32 midi, pUCN-N V_H1129 103-115 mini #3

	# colonies/plate	
pUCN-N/signal-V_H1129	#11	all white
pUCN-N/signal-V_L-huCkappa	>400	with light blue + white

1/9/91

picked 20 pUCN-N/signal-V_H1129 1-20
20 pUCN-N/signal-V_L-huCkappa 21-40

1-20) NcoI-PvuII
21-40) EcoRV-HinDIII



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4/5/91

Construction of chimeric signal- mV$_H$1129-huCκ in pUCN-N

Digested 100µl pUCN-N/32 with PvuII + NcoI
- 10µl React 2
- 4µl PvuII
- 4µl NcoI

Digested 36µl pUCN-N with Nco-SacI
- 4µl React 1
- 2µl NcoI
- 2µl SacI

Digested 10µl V$_H$1129 105-106 PCR fragment w/ PvuII + SacI
- 1.3µl React 4
- 1µl PvuII
- 1µl SacI

Preparative Gel to isolate pUCN-N/32 460bp PvuII-NcoI fragment

4/8/91

Digested 10µl huCκ PCR fragment with SacI + NotI
- 1.3µl React I
- 1.0µl SacI
- 1.0µl NotI

incubated 1.5 hr at 37°C.

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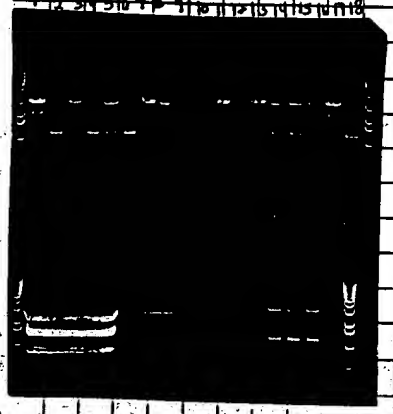
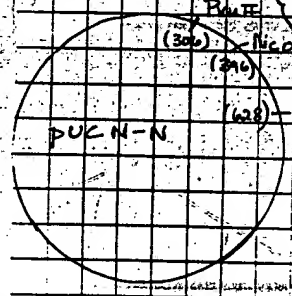
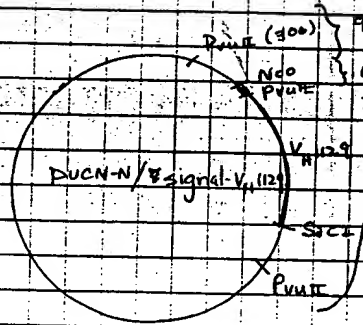
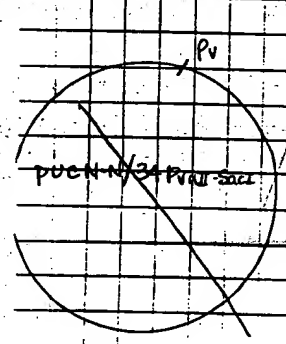


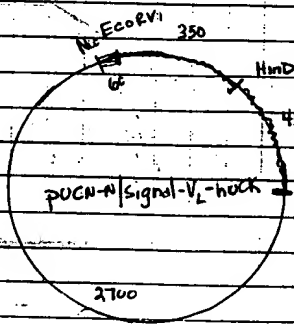
1-20) pUCN-N/signal-V_L-huCkappa-EcoRV-NotI
21) pUCN-N/34 EcoRV-NotI

A,B) pUCN-N/V_L-V_H #3 EcoRV-NotI
B) pUCN-N/V_L-V_H #2 EcoRV-NotI









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4/5/91

Construction of pUCN-N/V_L-huCkappa by overlapping PCR

Ligation

	pUCN-N V_L-huCk	pUCN-N control
pUCN-N EcoRI+NotI	3µl	3µl
V_L129-huCkappa EcoRI-NotI	5µl	–
H_2O	8µl	13µl
ligase Buffer (10X)	2µl	2µl
10mM ATP	2µl	2µl
Ligase	1µl	1µl

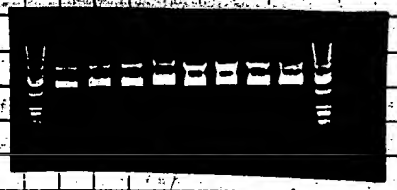
incubated in refrig. (4°c) over weekend

4-8-91

Transformed ligations into DH5-α

	# colonies/plate
pUCN-N control	150
pUCN-N/V_L-huCkappa	300 dark, medium blue + white

picked 48 blue colonies for mini preps 41-48



46 & 48 positives EcoRI-NotI digestion

#46 grown for midi prep.

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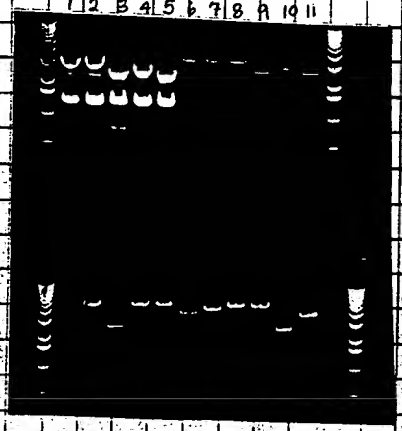
10/91

Completed midi preps of pUCN-N/V_L-V_H #2, #3 and pS436(110-111) #14, #15

1- pUCN-N/V_L-V_H #2 Sac
2- EcoRV
3- EcoRI-Bam
4- HinDIII
5- HinDIII-EcoRI
6- pUCN-N/V_L-V_H #3 SacI
7- EcoRV-Sac
8- EcoRV
9- EcoRI-BamHI
10- HinDIII
11- HinDIII-EcoRI
12- pS436(110-111) #14 NcoI
13- NotI
14- SalI
15- HinDIII
16- EcoRI-BamHI
17- #15 NcoI
18- NotI
19- Sal
20- HinDIII
21- EcoRI-BamHI



5µl of DNA loaded

? ?

looks good

EcoRII-Sac digestion of pUCN-N/V_L-V_H should generate an 0.8 kb fragment but appears to be only linearizing the plasmid. — Continue restriction digestion analyses to determine problem.

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4/9/91

Preparative gel to isolate huCγ1 SacI-NotI PCR fragment
pUC19-VJ/VL-VH1 EcoRV-SacI fragment
VH-huCγ1 103-116 EcoRI-NotI fragment

[redacted]

← huCγ1 SacI-NotI

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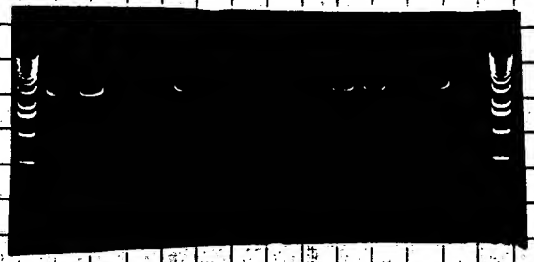
4-9-91

1- pS137 Not
2- pS137 Nco
3- pUC11-NV-V$_H$#3 EcoRV (React 4)
4- #3 EcoRV-Sac I
5- EcoRV-Not I
6- EcoRV-HinDIII
7- Cla
8- Cla-Sac I
9- Cla-Not I
10- Cla-HinDIII
11- EcoRV (React 2

do not cut



Digested 30µl pUC11-NV-V$_H$#3
4µl React 2
2µl Not I
2µl EcoRV
incubated at 37°C

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4-11-91

Ligations

	pUCN-N/34 control	pUCN-N/34 V_L-V_H #3	pUCN-N/34 V_L-V_H #2
pUCN-N/34 EcoRV-Not	2	2	2
V_L-V_H #2 EcoRV-Not f.	—	—	5
V_L-V_H #3 EcoRV-Not f.	—	5	—
Lig. Buffer	2	2	2
H_2O	14	8	8
Ligase	1	1	1
10mM ATP	2	2	2

refrigerated (4°C) until return

4/23/91

- Transformed DH5-α comp. cells with the above ligation mixtures
  - 1 hr. outgrowth
  - plated on LBAmp

- Transformed into DH5-α and plated on LBAmp + X-gal.

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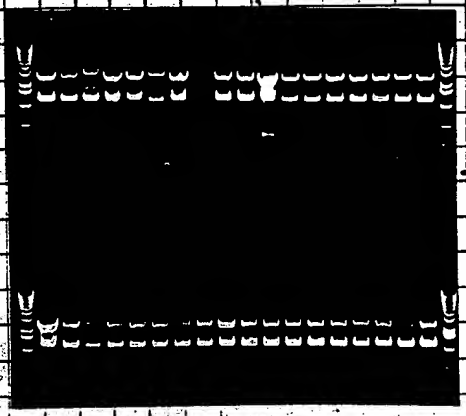
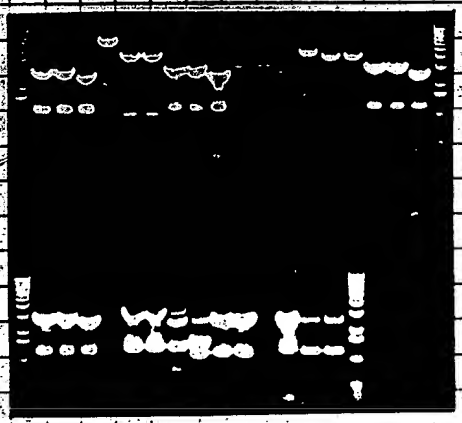


1-10) pUCN-N/V_L-huCKappa EcoRI-HindIII #1-10
11) pUCN-N V_L 103-104 EcoRI + HindIII
12-18) pUCN-N/S-V_L-huCKappa EcoRI + HindIII # 11-1
19) pUCN-N/V_L 103-104 EcoRI # HindIII
20)- 36) pUCN-N/S-V_L-huKappa #18-#34 EcoRI+H



1-	pUCN-N/34 Nco	19-	pUCN-N/V_L 103-104 HindI
2-	HindIII	20-	EcoRI
3-	Nco+HindIII	21-	HindI:Ec
4-	5)34 Nco	22-	pUCN-N V_L-V_H ~~103-104~~ Ec
5-	HindIII	23-	N
6-	Nco+HindIII	24-	Eco
7-	pUCN-N/34 (new) Nco	25-	pUCN-N EcoRV
8-	HindIII	26-	Not
9-	Nco+HindIII	27-	EcoRV+Not
10-	pUCN-N/32 Nco	28-	pUCN-N V_L 103-104 EcoR
11-	Sac	29-	pUNN S-V_L-huCKappa #35
12-	Nco+Sac	30-	" #36
13-	5)32 Nco	31	
14-	Sac		
15-	Nco+Sac		
16-	pUCN-N/32 Nco		
17-	Sac		
18-	Nco+Sac		

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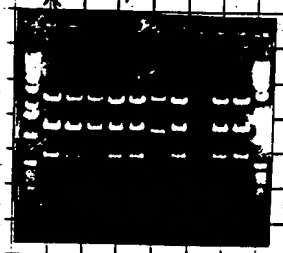
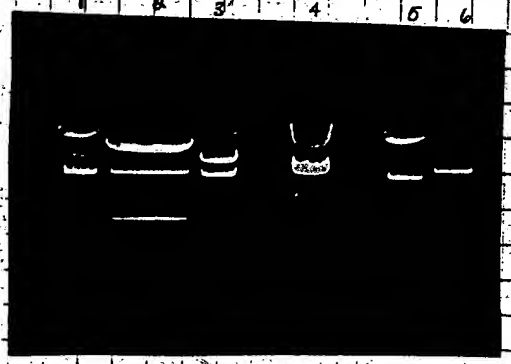


1-10) pUCN-N/V$_L$-huCKappa EcoRI + Not I

*

all but 4 and 8 are potentially correct
however, #3 is slightly bigger
should sequence several to determine if actually correct



1- pUCN-N/34 EcoRV-Not I
2- pUCN-N/32 Nco-Pvu II
3- pUCN-N/54 EcoRV-Sac
4- pUCN-N V$_L$-V$_H$ EcoRV-Sac
5- pUCN-N Nco-Sac
6- pUCN-N/mV$_L$-huCK (4+48) EcoRV-Not I

Gene Cleaned - pUCN-N/34 EcoRV-Not
pUCN-N/34 EcoRV-Sac
pUCN-N Nco-Sac

Freeze-squeeze
pUCN-N/32 Nco-Pvu II f.
pUCN-N/V$_L$-V$_H$ EcoRV-Sac f
pUCN-N/mV$_L$-huCK EcoRV-Not f.

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Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by	

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TITLE Book No.

From Page No. ~~4/26/91~~

4/30/91

6 Sequencing reactions

	Template		Primer
1-	pUCN-N/V_L-huCKappa	#46 midi	reverse ~~(5112)~~ UP
2-		#48 midi	"
3-		#1 mini	"
4-		#4 mini	"
5-		#48	5112
6-		#46	"
7-		#1	"
8-		#4	"
9-	//	#46	reverse (5112)
10-		#48	
11-		#4	

7

			Primer
1-	pUCN-N/S-V_H	#3 mini DNA	UP
2-		#4	"
3-		#5	"
4-		#7	"
5-		#8	"
6-		#9	"
7-		#10	"
8-		#11	"
9-		#16	"
10		#18	"

Notes: sequencing reactions on mini DNAs did not work
∴ probably not enough DNA

Grew midi cultures (50ml) of pUCN-N/V_L-huCk #1, #4
pUCN-N/S-V_H #4, 5, 9, 18

pUCN-N/V_L-huCKappa #46 & #48 were blue; sequencing showed 46 co
a two-base-pair deletion in linker region & 48 has a one base pair Δ in linker. To Page

Witnessed & Understood by me,	Date	Invented by Rose L Bennett	Date 5/1/91
		Recorded by	

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5/1/91

Digested 36µl of pS141 (pUCN-N/34) with ~~HindIII~~ EcoRV and NotI

4µl React 2

2µl EcoRV

2µl Not

Incubated for 1hr. at 37°C.

Fractionated on 1% agarose gel O/N

To Page

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From Page No. 5/6/91

8 Developed the following sequencing gel

1- #4
2- #5
3- #9 } S-V_H 1129 potential clones UP primer ?
4- #18

5- #1 UP
6- #1 S.112 mV_L 1129 - huCkappa ⇐ sequence is correct
7- #1 reverse

8- #4 UP ?
9- #4 S.112 mV_L 1129 - huCkappa
10 #4 reverse

To Page N

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		Recorded by	

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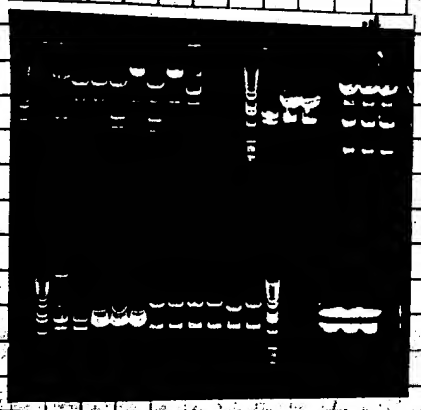

1- ~~ps~~ MW std
2- ps134 uncut
3- EcoRV
4- RV-HinDIII
5- RV-HinDIII
6- HinDIII
7- NcoI
8- EcoRV-SacI
9- SacI
10- NotI
11- ps140 uncut
12- HinDIII
13- Nco
14- ps134 Nco+HinDIII

15- ps141 uncut
16- EcoRV
17- EcoRV
18- EcoRV-Sac
19- Sac
20- EcoRV-HinD
21- HinD
22- RV-Not
23- Nco Not
24- Nco-Not
25- Nco
26- ps140 HinDIII-Nco

Note: ps141 does not appear to have an EcoRI site
ps134 however has two ~~five~~ NcoI-HinDIII fragments of about 400bp
therefore ps141 probably contains the wrong Nco-HinDIII frag
Will redo the construction and screen miniprepe with EcoRV+Hin

Isolated ps134 Nco+HinDIII 400 bp fragments
ps140 Nco+HinDIII 2.5 Kb fragment

Ligations	ps140 Nco-HinD	ps140 Nco-HinDIII
ps140 Nco+HinDIII 2.7 Kb f.	1µl	1µl
ps134 Nco+HinDIII 400bp fragments	6µl	—
10X Ligase Buffer	2µl	2µl
10mm ATP	2µl	2µl
H_2O	6µl	14µl
Ligase	1µl	1µl

incubated 12 hr. at room temperature.
transformed into comp. DH5-α (Sid's cells)

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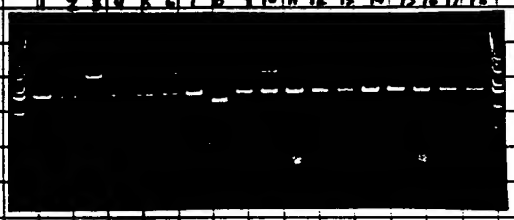
Construction of mV_H - huX minigene

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18



1-18) potential pS14D $S-V_H$ 129 Neo - PvuII

No clones appear to be correct

Result: This three-part ligation strategy is not working Need a new plan

To Page

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om Page No.

Sequence (Triplets): CCC AAT TCC CAC TCA TTT ACC
CGG AGA CAG G
SJ21 1.39 mg/ml
human Cκ 3' primer

Sequence (Triplets): GTC ACC GTG AGC TCA GCC TCC
ACC AAG G (Sac I)
Cκ 5' primer
SJ22 1.99 mg/ml

Sequence (Triplets): CGG AAT TCA GGT TTA TCT GCA
GTA GTC WGG ([A/T])
SJ42 2.66 mg/ml

Sequence (Triplets): CGG AAT TCA GGT SMA RCT GCA
([C/G])([A/C])([A/G])
GSA GTC WGG
([C/G]) ([A/T])
SJ43 1.72 mg/ml

Sequence (Triplets): CGG AAT TCA GGT [illegible] GCT GCA
GCA
GCA GTC TGG
SJ44 2.92 mg/ml

Sequence (Triplets): 10 20
GGGAATTCC ATGGACTGGA CCTGGAGGGT C
5' section AB of humanized 1308F V-H
SJ34 2.033 mg/ml

Sequence (Triplets): GGC GGA TCC GAG GTG CAG CAG
CTG GTG CAG
257 V_H '5 (PvuII)
SJ71 3.96 mg/ml

To Pag N

Recorded by [signature] 5/8 94

TITLE _____ Project No. _____ Book No. _____

From Page No. 5-8-91

Isolation of 447 (H^{VI}_{gp41} Mab) from mouse-human hybrid cell line.

A. Isolated poly A^+ RNA (mRNA) using in vitro Micro Fast Track kit -see instructions w/ kit.

B. Generated cDNA from 1/3 volume poly A^+ 447 mRNA 7µl -see kit instructions

C. Ethanol ppt washed and resuspended in 20µl volume H_2O phenol extracted

D. PCR Reaction using the following primer combinations:

Tube	Template	Primer
A	447 cDNA	SJ21
		SJ73
B	"	SJ21
		SJ92
C	"	SJ2
		SJ43
D	"	SJ21
		SJ44
E	"	SJ21
		SJ84
F	"	SJ2
		SJ88
G	"	SJ2
		SJ00
H	"	SJ03
		SJ00

[redacted]

Template DNA	Volume 0.5µl
10X PCR Buf	10µl
10X dNTP	10µl
Primer #1	1µl
Primer #2	1µl
Taq Pol	1µl
H_2O	67.5µl

Also wanted to do rxn with SJ21 + SJ71 but could not find SJ71.

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Witnessed & Understood by me, Tessa J. Bennett

Date 5/8/91

Invented by

Recorded by

Date

Sequence (Triplets): CAG GTG CAG CTG
CAG GTG TCG GGC CC
V_H 248 (5'-Pst PvuII)
SJ73 2.49 mg/ml

Sequence (Triplets): GGG AAT CGA GCT CGT CAT GAA
ACA CCT GTG G[redacted]
T SJ82 4.64 mg/ml
BspHI at natural VH signal of 98-6

SJ119 Hu1308VH/H71 arg to ser (top)
5' AgA gTC ACC ATg ACC TCA gAC ACg TCC ACg AgC

SJ120 Hu1308VH/H71 arg to ser (bottom)
5' gCT CgT ggA CgT gTC TgA ggT CAT ggT gAC TCT

SJ121 Hu1308VH/mu to hu 66-71 (top)
5' AAg gCC AgT ATT ACA TCA gAC ACg TCC ACg AgC

SJ122 Hu1308VH/mu to hu 66-71 (bottom)
5' gAT gTA ATA CTg gCC TTg CCC Tgg AAC TTC ggg

SJ123 Human C-gamma 3' / RI, NotI
5' CCg AAT TCg Cgg CCg CAC TCA TTT ACC Cgg AgA CAg g

Witnessed & Understood by me, Date 5/8/97 Invented by Date
Recorded by

Project No.

TITLE Book No.

From Page No. 5-9-91

Construction of pSJ40 containing pSJ34 NCo-A HinDIII fragment

1- uncut 40-34 #3
2- 40-34 #3 EcoRV
3- #4 EcoRV
4- #5 EcoRV
5- #6 EcoRV ✓
6- #7 EcoRV
7- #9 EcoRV ✓
8- pSJ34 EcoRV
9- pSJ40 HinDIII

Using LBAmp + 5% X-gal, showed that clone #7 turns blue while potential positive clones #6 and #9 remain white. This result is the opposite of what was expected. The HinDIII and the SacI sites are supposedly in frame with lacZ.

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Lisa J. Bennett	5/8/91	Recorded by	

ject No.

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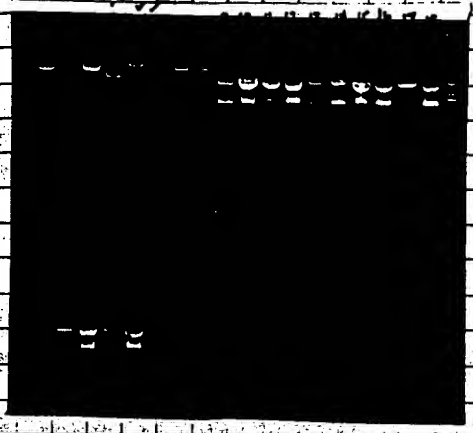
From Page No. 5-14-91



1- pSJ32 Nco-Sac
2- "
3- pSJ34 RV-HinD
4- "
5- pSJ36 BamHI-EcoRI
6- "
7- pSJ37 Nco-EcoRI
8- "
9- pSJ40 Nco
10- "
11- pSJ42 Nco-Sac
12- pSJ42 Nco-Sac
13- pSJ44 HinDIII
14- pSJ44 HinDIII
15- pSJ45 EcoRI-HinDIII
16- "
17- pSJ46 Sal I-BamHI
18- "
19- pSJ47 EcoRI-Bam
20- "
21- pSJ48 EcoRI-Bam
22- "

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Witnessed & Understood by me, Lisa Bennett

Date 5/14/91

Invented by

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Date

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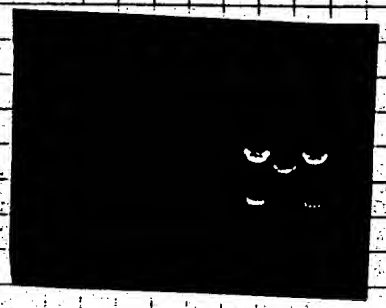
1- pS141-6 EcoRI
2- EcoRI-HindIII
3- HindIII
4- Nco-HindIII
5- Nco
6- Not
7- EcoRV-NotI
8- EcoRV
9- pS141-9 EcoRI
10- HindIII
11- Nco-HindIII
12- Nco
13- Not
14- RV-NotI
15- EcoRV
16- pS143 EcoRV
17- EcoRV-NotI
18- NotI
19- pS143 EcoRV
20- EcoRV-Not
21- Not



Preparative gels

1- pS141-6 EcoRV-Not no DNA
2- pS141-9 EcoRV-Not very dilute
3- pS143 EcoRV-Not isolated
4- V_H1129 PvuII-SacI

To Page

Witnessed & Understood by me, Susi Bennett Date 5/14/91

Invented by Date

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om Page No.__ 5-15-91

Ligations

	41 control	41 43	41 49	V_H – pSJ32(0.4)	V_H pSJ32(0.8)
pSJ41 EcoRV-NotI f.	2μl	2μl	2μl	–	–
pSJ43 EcoRV-NotI f.	–	5μl	–	–	–
pSJ49 EcoRV-NotI f.	–	–	5μl	–	–
pSJ32 BglI-PvuII, BglI-SacI f.	–	–	–	2μl	2μl
V_H 1129 0.4 BPvuII-SacI	–	–	–	5μl	–
V_H 1129 0.8 PvuII-SacI	–	–	–	–	5μl
Lbuffer	4μl	4μl	4μl	4μl	4μl
Ligase	1μl	1μl	1μl	1μl	1μl
H_2O	13μl	8μl	8μl	8μl	8μl

incubated at RT for 3 hr
transformed into DH5-α, plated on LBAmp

5-16-91

Pick 6 pSJ32 V_H (0.4)
6 pSJ32 V_H (0.8)
~~41-43~~
12 41-43
12 41-49
} grew 5ml minipreps

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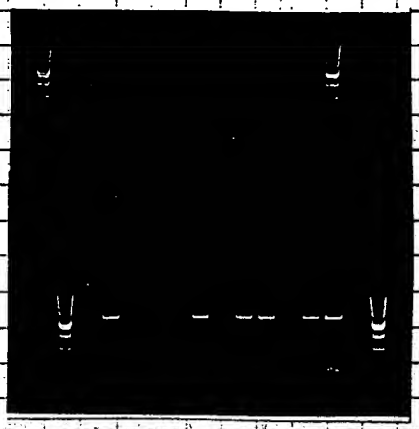
From Page No. 86 5-17-91

Digestion of minipreps

1- S-V_H (0.4) #1-6 Nco-Sac
2- S-V_H (0.8) #7-12 Nco-Sac
3- 41-43 #14
4- #16
5- #18 } Nco-SacI
6- #20
7- #21
8- 41-49 #25
9- #26
10- #29
11- #31
12- #33 } Nco-NotI
13- #34
14- #35
15- #36



1- pS-V_H #1 BglI-Xho
2- #2 "
3- #3 "
4- #4 "
5- #5 "
6- #6 "
7- pST32 "

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		Recorded by	

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Book No._____ TITLE _____

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2/1/91

Transformed miniprep samples #1, #2, #6, #18, #21, #26, #33 into HB101 Comp. cells
Plated on LBAmp.

2/8/91

1- S-V_H #1 Nco-PvuII
2- " #2 "
3- S-V_H #6 "
4- S-V_H #1 BglI-XhoI
5- S-V_H #2 BglI-XhoI
6- S-V_H #6 BglI-XhoI
7- 41-43 #18 Nco-SacI
8- 41-43 #21 Nco-SacI
9- 41-49 #26 Nco-Not
10- 41-49 #33 Nco-Not

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tnessed & Understood by me,	Date	Invented by	Date
		Recorded by	5/21/92

I I I

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TITLE Book No.

From Page No. 5/21/91

PCR Reaction to amplify huCκ1

Template	Fragment	Primers	Program
268 SD.125mp	huCκ1	ST22	94°C 2min
		ST123	94°C 1min, 52°C 2min, 72°C 2min } 30

[redacted]

phenol: chloroform, chloroform, ethanol ppt, ethanol wash
digest with SacI and EcoRI in React 4
digest pUC18 with SacI and EcoRI

5/22/91

[redacted] preparative gel of huCκ1, EcoRI-SacI
isolated and Gene Cleaned

[redacted] pUC18 EcoRI-SacI
isolated and Gene Clean

To Page

Witnessed & Understood by me,	Date	Invented by Lisa Burdett	Date 3/24/91
		Recorded by	

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Book No. ____ TITLE ____

m Page No. 5/23

igations

	pUC18	pUC18/huCK$_1$
pUC18 EcoR1-Sac	2 µl	2 µl
huCK$_1$ EcoR1-Sac PCR f.	—	5 µl
H_2O	13 µl	8 µl
Buffer	4 µl	4 µl
Ligase	1 µl	1 µl

Incubated at RT for 2 hr.

ransformed into DH5-α and plated on LBamp

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nessed & Understood by me, | Date | Invented by [signature] | Date 5/23/91

Recorded by

P 'ect No._____

TITLE_____ 'ook No._____

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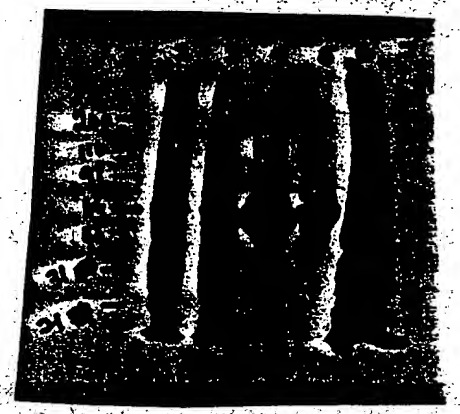
Western blot

		amount
1- MW std.		
2- V. SH CS # 4	(neg. control)	10µl -
3- dbr FR	positive control	10µl -
4- RSV long sup		15µl
5- RSV Hep2 sup		15µl
6- HEP2 sup		15µl -
7- HEP2 pellet		15µl



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		Recorded by	

Exhibit 3

David S. Pfarr Notebook References for Humanized IgG Expression

Expression Vector Construction

Notebook	Procedure	Pages	Date
87	MIMEV (pMI301) Expression Vector Construction	1-24; 41-44; 50-56; 67; 71-87;	6-7-91

Table of Contents

TITLE pSJ36 1/ Project No.

Book No. 87

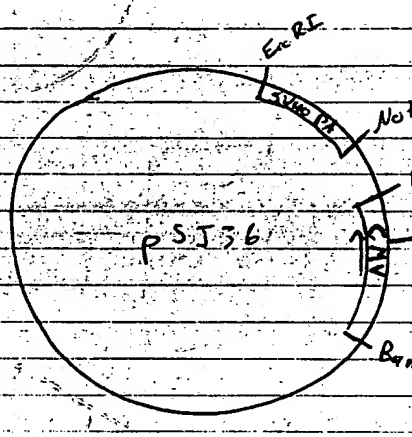
From Page No. pSJ36 Nco knockout / MCS insertion
(pSJ37 revisited)

7 June 1991

Rec'd pSJ36 DNA (50ul) and linkers SJ110, SJ111 from Syd Johnsen; to 4°C o/N.

10 June 1991

Cut pSJ36 ε NcoI: 16.5 λ H_2O
2.5 λ NEB 4
5 λ pSJ36
1 λ (25U) NcoI
~37°C @ ~8:15 AM



SJ110 - (Hind III),NcoI,NotI,SalI adapter - top
5' (p)AgC TCC ATg gTC ggC ggC CgCg g
Hind 3 NcoI NotI Sal I

SJ111 - bottom
5' (p)TCg Acg Cgg CCg CCg ACC ATg g
Sal I

Digest out @ 10:45: 1% Agarose / TBE gel

BRL 1Kb ladd.
1 λ Digest
.5 λ pSJ36

To Pag

Witnessed & Understood by me,	Date	Invented by / Recorded by	Date
			11 June 1991

Project No.
Book No. 87 TITLE pSJ36/11
Page No.

ppt DNA (5λ 10M NH4OAc, 65λ EtOH, Spin 10')
decant, dry, resuspend in 20λ H_2O.

Fill in NcoI ends:

20λ DNA
2.5λ 10X Klenow Buffer (Shirley Huan)
2.5λ 2mM dNTPs
0.54λ (3 U) Klenow
-- 37°C 3^{30} - 4^{50}.

Add 1μl .5M EDTA, 65°C 5', EtOH ppt.

Anneal linkers SJ110/SJ111 (@ 1mg/ml):

2λ SJ110 (2μg)
2λ SJ111 (2μg)
~~10~~λ 5X BRL ligase Buffer ([$MgCl_2$] final = 2.5mM)
1λ 5M NaCl ([final] = 50mM)
85λ H_2O
-- 65°C 20', remove in 400ml H_2O/600ml beaker.
let cool to Room Temp.

Dry pSJ36Nco/fill in, resuspend in 20λ, ligate:

19 ~~20~~λ DNA
5λ 5x BRL ligase Buffer
1λ ligase
-- 16°C O/N

June 1991

ppt Ligation, spin, dry, resuspend in 18.5λ H_2O.
Cut c̄ NcoI: 17.5 ~~18.5~~λ DNA — 1λ for gel
2λ NEB4
.5λ (12.5U) NcoI

T Page No.

Witnessed & Understood by me. | Date | Invented by DJS Pfau | Date 6/11/91
Recorded by

pSJ36N

Project No. ____
Book No. 87

Digest @ 37°C 8:45 AM, out @ 9:__

1% Agarose Gel: ① 250ng λH3
② SJ36 NcoI fill In
③ SJ36 NcoI fill In, religated
④ SJ36 NcoI fill in/religated/NcoI recut

can see NcoI fill in, but nothing in the other 2 lanes.

Digest to 65°C 5', on ice, xform 10ul into Hanahan competent (J. Berrios) HB101, along with 1ul pSJ as a control.

plate 2×100ul and 100ul 1:10 of pSJ36N (Tvo...
plate 100ul 1:10 pSJ36 (Tvol = 600ul).

-- 37°C o/N

pSJ 110/111 : AGCTCCATGGTCGGCGGCCGCG
GGTACCAGCCGCCGGCGCAG

HindIII NcoI NotI SalI

These adapters have a real bad Kozak
T in the -3 position, which is the least f...
base in that position.

-- Toss SJ110/111 annealing rxn.

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Witnessed & Understood by me. | Date | Invented by [signature] | Date 6/14/91

Recorded by

Project No.

Book No. 97 TITLE pSJ36N⁻

Page No.

June 1991

Transformation: pSJ36 control (100λ 1:10 = 1.7% of transformation) gave 19 colonies.

pSJ36N⁻: 100λ (17% of transformation) gave 46 and 37 colonies; 100λ 1:10 (1.7%) gave 2 colonies.

Pick 12 colonies to 4ml LB-tmp; 37°C shaker.

1[30]: Spin cultures, mini-preps (LiCl), resuspend in 50λ TE.
4λ each to .8% agarose/TAE gel:
note: bottom of wells must have pulled up, because sample won't not enter wells very easily; lost 25%-50% of each sample.

[redacted]

Can't tell much, DNAs mostly running as open circles, #7, 8 look OK, ∴ all are probably ~ right size.

Inoculate 50ml cultures of 6, 7, 8; 37°C o/n.

ssed & Understood by me,	Date	Invented by D. S. Haas	Date 6/17/91
		Recorded by	

T Page N

TITLE pSJ36N

Project No.

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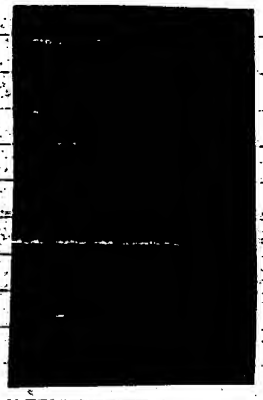
From Page No.

13 June 1991

Cut 5 λ # 3, 4, 5, 6, 7, 8 ⊂ NcoI: 5 λ DNA
1 λ NEB4
3 λ H_2O
1 λ (Sv) NcoI
--37°C @ 7³⁰ pack@

1% Agarose/TBE Gel:

#1	#2
λ H3	λ H3
SJ36 NcoI	SJ36 S.C.
3 S.C.	6 S.C.
3 NcoI	6 NcoI
4 S.C.	7 S.C.
4 NcoI	7 NcoI
5 S.C.	8 S.C.
5 NcoI	8 NcoI



None cut ⊂ NcoI, but that could simply be to dirty DNA, etc.

Innoculate 100ml LB-AMP cultures with # 6, 7, 8. 37°C o/N.

14 June 1991

Alkaline lysis preps of 6, 7, 8; EtOH/CHCl₃ x1
CHCl₃ x1, ppt 2x.

Resuspend in 200ul TE; to 4°C.

To Pag

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by	6/17/91

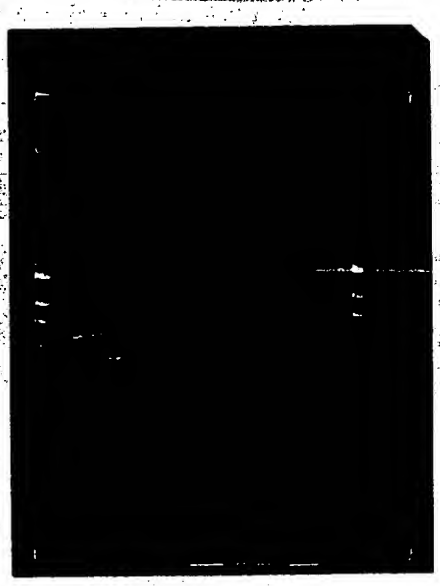
Book No. 87 TITLE pSJ36N⁻

Page No.

7 June 1991

Cut #6, 7, 8 ⊂ NcoI, PvuI, NcoI/PvuI:

7λ H_2O
.5λ BRL 3
.5λ BRL 7
1.5λ DNA
.5λ Enzyme
37°C @ 10:30 to 11:. 1% agarose/TBE:



-- pSJ36 definitely cuts ⊂ both enzymes.

-- #6, 7, 8 all seem to linearize with NcoI, PvuI, and Nco/Pvu, but they don't drop out the smaller Nco/Pvu band. (?)

-- #7 still shows mostly (all?) supercoiled in the NcoI lane.

Pick #7 as pSJ36N⁻.

June 1991 Cleanup pSJ36N⁻ #7: qOH/$CHCl_3$ x 2, $CHCl_3$ x1, ppt

Resuspend in 400μl TE, ppt. o/N.

June 1991 Spin DNA, 70% wash, Dry, resuspend in 200μl TE.

T Page No.

ssed & Understood by me,	Date	Invented by	Date
		Recorded by Dan S. Gluck	19 June 1991

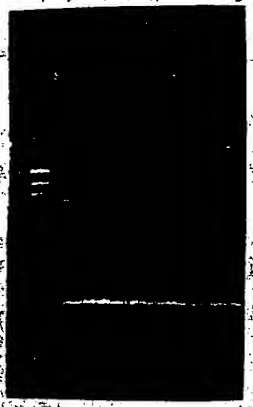
pSJ36/11

Book No. 37

Cut pSJ36/11 with NcoI, PvuI, NcoI/PvuI to assure NcoI site is gone:

7.5λ H_2O
1λ DNA
1λ 1.1 NEB3/NEB4
.5λ enzyme
-- 37°C 10^2 out 11^42.

1% Agarose/TBE gel:



PvuI cuts in β-lactamase of pBR region, and both NcoI and PvuI cut the plasmid it shou cut into 2 smaller (~3K, 1.6K) bands.

PvuI does cut, NcoI does not.

To Pa

Witnessed & Understood by me. | Date | Invented by D. S. Robb | Dat 19 June 199

Recorded by

Project No.____
Book No. 7 TITLE pSV∆2 DHFD

Page No.

3) λ ~~BamH I~~ HindIII DJ 7-1-91 /MB / Pvu II to 1% Agarose/TBE gel:

[redacted]

Digest looks good -- lg. fragment migrates at a lower mobility, and can see the small SV40 promoter/enhancer fragment.
-- to 4°C O/N.

27 June 1991 Run DNA on .8% Agarose/TBE Prep gel.
-- cut out large (4.1 kb) band, GeneClean elute, ppt O/N.

28 June 1991 spin, dry, resuspend in 50λ TE
Run 1λ on 1% Agarose/TBE:

[redacted]

DNA looks good, clean.
~50 ng/λ

July 1991 Rec'd SV40 Early promoter PCR primers:
5'→3' = DP-P107: CCG·GAA·TTC·ACG·CGT·AGT·CCC·GCC·CC
@ 1.66 ug/ul (117 pmoles/ug) = 194.2 pmols/ul T_m = ~8
3'→5' = DP-P108: GAA·GCG·CTC·GAG·CAA·AAG·CCT·AGG·CCT·CC
@ 2.54 ug/ul (104 pmols/ug) = 264.2 pmols/ul T_m = ~9

dilute: DP-P107 to 2.5 uM: 6.44 ul (1251 pmole) → 500 ul TE
= 2.5 pmol/ul

dilute: DP-P107 to 2.5 uM: 4.73 ul (1250 pmole) → 500 ul TE
= 2.5 pmol/ul

dilute: pSV2DHFRNeo #3 (P/6) 1:500 in TE.

T Pag N

essed & Understo d by me,	Date	Invented by / Recorded by	Date
		[signature]	7/1/91

TITLE pSV2 DHFR Neo T-

Project No.

Book No. 87

From Page No.

10λ DNA
3λ 5X ligase
1λ H_2O
1λ (1U) ligase
-- RT @ 7 to 1M

Xform into HB101, ± 1λ pSJ36 as control xformation, after expression, spin, resuspend in .7ml, plate 2x. of ~~pDHFR~~ pSV2DHFRNeo.

20 June 1991 No xformants in pSV2DHFRNeo plates, very ~ on pSJ36 plates. Toss all.

24 June 1991 Cut pSV2DHFR and pSV2Neo with NcoI:
7.5λ H_2O
1λ NEB4
.5λ DNA
1λ (5U) NcoI
-- 37°C @ 7:20 1M to 8:40 1M.

Partial NcoI cut pSV2DHFR: 7λ H_2O
1λ NEB4
2λ (4.8mg) DNA
.5λ (25U) NcoI
-- 37°C 7:20, out at 8:00
-- add 1λ .5M EDTA, 65°C 5'; remove for gel.

1% Agarose/TBE gel:
Lane 1: pSV2DHFR S.C.
2: pSV2DHFR Neo Partial
3: pSV2DHFR Neo Complete
4: pSV2Neo Neo complete
5: λ H3

T Pag

Witnessed & Understood by me.	Date	Invented by / Recorded by	Date
		[signature]	24 June 1991

Project No._____
Book No.__ __ TITLE pSV2 DHFR-like

Page [redacted]

Both pSV2DHFR and pSV2Neo have 2 NcoI sites.

pSV2DHFR NcoI partial digest looks good.

ppt, 70% wash, dry. Resuspend in 23λ H_2O, cut in:

23λ DNA
3λ 10x Klenow Buffer
3λ 10x dNTPs
1λ (6U) klenow
--37°C 30'

ppt, dry, resuspend in 22λ H_2O, cut: NcoI:

22λ DNA
2.5λ 10x NEB4
.5λ (17.5U) NcoI
--37°C @ 4:35 PM.

5:40 PM: Add -- 17λ H_2O
2.5λ 10x Klenow Buffer
5λ 10x dNTP's
.5λ (3U) klenow
--37°C 30'
ppt. O/N.

June 1991

Spin DNA, 70% wash, dry, resuspend in 20.5λ H_2O.
Ligate:
20.5λ DNA
2.5λ 10x Ligase Buffer
1λ 50mM ATP
1λ ligase (1U)
--Room Temp 1hs.
--add 1λ ligase, RT 1hr.

T Page N__

ssed & Understood by me,	Date	Invented by / Recorded by	Date
		D. S. Pfau	25 June 1991

Book No. R7 TITLE pSV2DHFR-11-0-

Page No.

1 July 1991

ppt pSV2DHFR Nco⁻ #3, spin, dry, resuspend in 100λ TE.
--add 400λ Elutip-D Low Buffer, run on Elutip-D Column
① elute, ppt, dry, resuspend in 50μl TE.

July 1991

Cut pSV2DHFR Nco⁻ #3 with PvuI, NcoI, PvuI/NcoI:

NcoI cuts! Toss DNA.

Already used DNA for PCR, so will subclone the PCR fragment and knock-out the NcoI site in that vector (see p. 17-18).

[redacted]

T Page N

sed & Understood by me. | Date | Invented by D. S. Ryan | Date 7/5/91
Recorded by

TITLE pSV2DHFR

Book No. 87

From Page No. 10

Set up PCR reaction:

- ✓ 10ul 10X PCR Buffer
- ✓ 10ul 10X dNTP
- ✓ 10ul 2.5uM DP-P107
- ✓ 10ul 2.5uM DP-P108
- ✓ 58ul H_2O
- ✓ 1ul pSV2DHFR Neo⁻ 1:500
- ✓ .5ul (2.5U) Taq Polymerase

PCR File (84) 96°C/3' x1

(86) 96°C/45", 72°C/45" x30

(11) 8°C hold

2 July 1991

Rxn. out at 7:21 PM; load 10ul (10%) on 2% agaros
-gel on P. 22

No PCR Product Srtn.

Set up new PCR Rxn - same as 7/1 (above)

DSP 7/2

PCR File (84) 96°C/3' x1

(86) ~~96°C/1'; 68°C/1'; 72°C/1' x30~~

(11) 8°C hold

Note: Tm for region of complementarity to SV40:
DP-P107: 42°C
DP-P108: 58°C

Total Tm:
DP-P107 = 88°C
DP-P108: 94°C

To Page N

Witnessed & Understood by me,	Date	Invented by / Recorded by	Date
		[signature]	7/2/91

Project No.

Book No. 87 TITLE pSVA2 DHFR

Page No.

PCR File

(84) 96°C, 3' x1

↓

(92) 92°C, 1'/42°C, 1'/60°C, 45" x5

↓

(86) 96°C, 1'/66°C, 75"/72°C, 45" x25

↓

(11) 8°C hold

Run 10λ (10%) on 2.0% Agarose/TAE gel:

--5 hrs to -20°C.

SV40 promoter band looks good!

July 1991

Phenol/$CHCl_3$ x1; $CHCl_3$ x1; Ppt; spin; wash; dry.

Resuspend in 20.5λ H_2O; cut Eco RI:

✓ 20.5λ DNA

✓ 2.5λ BRL 3

✓ 2λ (100U) Eco RI

--37°C @ 10:25 AM; out at 1:55 PM.

--to -20°C

2 July 1991

Add: ✓ 2.5λ BRL 3

✓ 18.5λ H_2O

✓ 3λ (45U) Eco 47III

✓ 1λ (50U) Eco RI

--37°C @ 10:15 AM.

T Pag No.

ssed & Understood by me.	Date	Invented by / Recorded by	Date
		D. J. S. Pfau	7/10/91

TITLE pSVΔ2DHFR

Project No.
Book No. 87

From Page No.

2[35] PM: add another 2ul (30U) Eco47III and 1ul (50U) EcoRI
-- to 37°C o/N

11 July 1991

Ppt SV40Δ PCR RI/47III digest; spin; dry; resuspend 25λ TE.

Fill in ends: ✓ 25λ DNA
✓ 3.5λ 10x klenow buffer
✓ 3.5λ 10x dNTP's
✓ 2λ H_2O
✓ 1λ klenow
-- RT @ 10[00] to 10[60] 1/M
-- add 1λ .5M EDTA, 65°C 10'

Set up ligation: ✓ 4ul pSV2DHFR Bam FI/HG/PvuII
✓ 5ul pSV40Δ promoter PCR RI/Eco47III f.
✓ 3ul 10x ligase buffer
✓ 1ul 50mM ATP
✓ 16ul H_2O
✓ 1ul Ligase
-- 16°C @ 12[00]

12 July 1991

xform 12ul into HB101; plate on 3 plates

13 July 1991

no colonies in transformation plate.

17 July 1991

Xform 5ul ligation into 13ul ME-DH10; plate all 2 plates

18 July 1991

Many colonies (TNTC); pick 12 to LB-Amp @ 7[2]

3[20] PM: alkaline lysis preps; to -20°C o/N.

T Pa

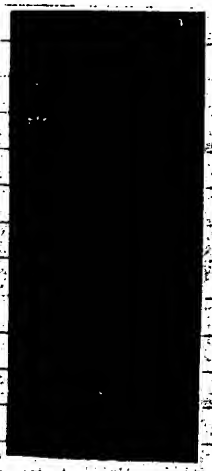
Witnessed & Understood by me,	Date	Invented by / Recorded by	Date
		[signature]	7/18/91

Project No. _____
Book No. 87 TITLE bFH PCR Fragment

Page No. _____

July 1991

Rxn. out at 7:00 AM. Run 10ul (10%) on 2% Agarose/TAE Gel:



No bands seen.

Set up new RXN; same as 7/1 (PZ1)

PCR File: (84) 96°C/3' x1 ~~DSP.7Iz~~

(86) 96°C/1', 66°C/1', 72°C/1' x30

(11) 8°C hold

Note: for region of complementarity to bFH, T_m's =

DP-P105 : 50°C

DP-P106 : 58°C

Total T_m =

DP-P105 : 90°C

DP-P106 : 96°C

PCR File (84) 96°C, 3' x1

↓

(82) ~~92~~ 94°C, 1'/42°C, 1'/60°C, 45" x5

↓

(86) 96°C, 1'/66°C, 45"/72°C, 45" x25

↓

(11) 8°C Soak

Run 10λ (10%) on 2% Agarose/TAE gel:

To Page No. _____

ssed & Understood by me,	Date	Invented by D. S. Rafan	Date 7/2/91
		Recorded by	

TITLE _____ Project No. _____ Book No. 87

From Page No. ___

Gel on P18 -- can see ~1500 bp fragment

5 July 1991

Set up PCR RXN:
- ✓ 10 ml 10x PCR Buffer
- ✓ 10 ml 10x dNTP's
- ✓ 10 ml 2.5 mM DP-P105
- ✓ 10 ml 2.5 mM DP-P106
- ✓ 58.5 ml H_2O
- ✓ .5 ml DSP1.1 BGH-T 1:1000 (= ~1 ng DNA)
- ✓ .5 ml Taq Polymerase

PCR File

84 (DSP) 94°C, 45" / 42°, 45" / 72°C, 45" x5

86 96°C, 45" / 60°C, 45" / 72°C, 45" x25

11 8°C Soak

--Run 10λ (10%) on 2% agarose/TBE!

Can possibly see a faint ~200 bp band. To -20°C.

8 July 1991 ΦOH/$CHCl_3$ x1, $CHCl_3$ x1, ppt, resuspend in 20λ H_2O.

Tos/s PCR RXN!!

DP-P105 (TGC·TCG·ACG·TGC·ACG·GTC·GGT·AGA·CA
5' SalI Rev. PmlI

is wrong!

--only have a partial SalI site and the PmlI site is backwards -- should be CAC GTG

Will redesign this primer and start over!!

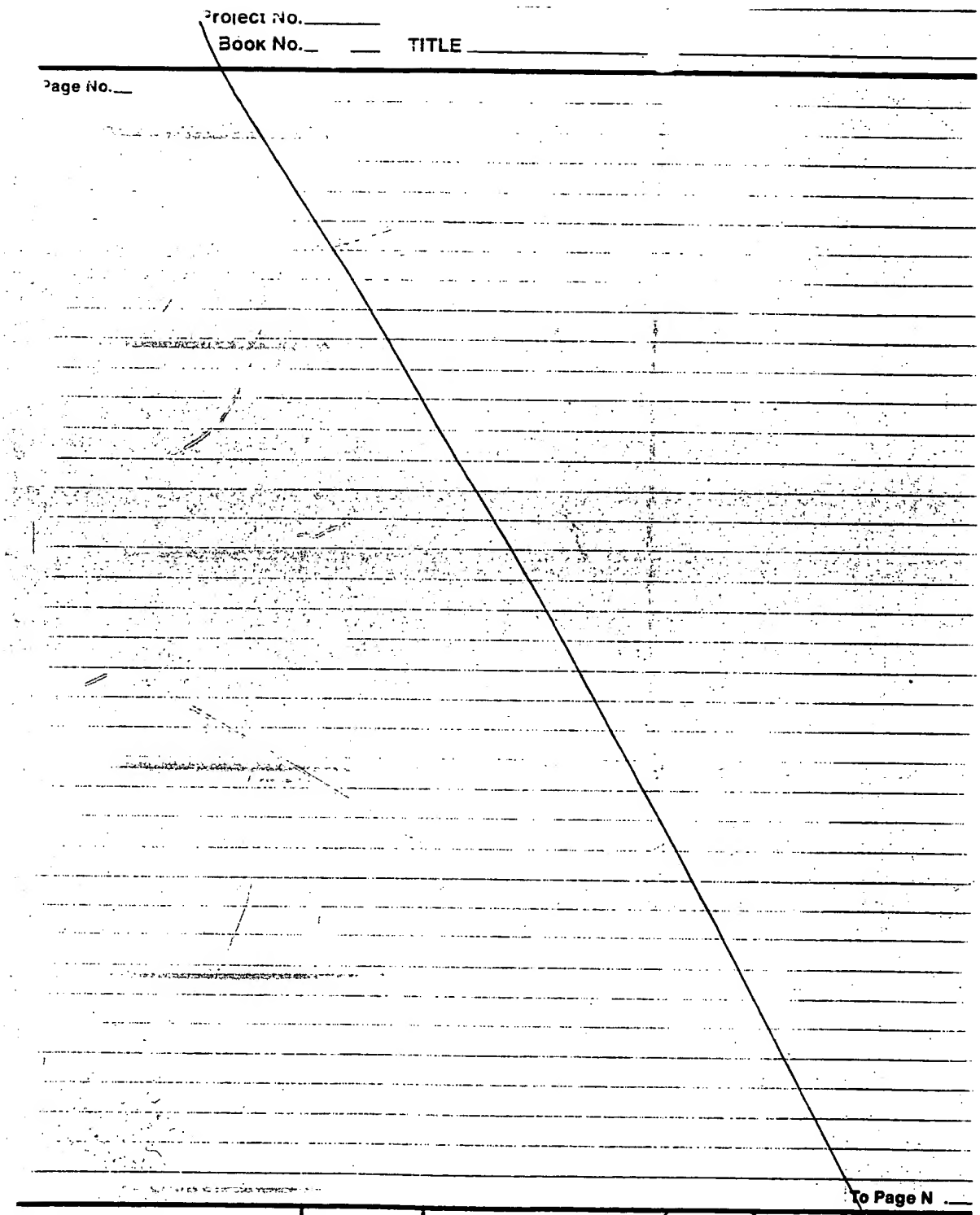
T Page

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by	7/8/91

Project No. _____

Book No. ___ TITLE _____

Page No. ___



To Page N ___

essed & Understood by me,	Date	Invented by	Date
		Recorded by	7/8/91

TITLE pSJ36N⁻-L   Project No.____ Book No. 87

From Page No.__

pSJ36N⁻-L1

8 July 1991 Kozak linker insertion into pSJ36N⁻.

Cut pSJ36N⁻ with SalI: 20µl DNA
10µl React 10
65µl H2O
5µl (50U) SalI
-- 37°C @ 1⁴⁰ PM

~~Anneal DP-L101/DP-L102 linkers:~~
~~BamHI~~
~~L101 GAT·CCG·GTC·CGT·CTA~~
~~L102 GC·CAG GCA GAT·CTA·G~~
DSP 7/8/91

Anneal DP-L103/DP-L104 linkers:
HindIII overhang; Knock out #3 — NcoI — NotI
DP-L103 AGC·TAG·CCG·CCA·CCA·TGG·TCG·GCG·GCC·G
DP-L104 TC·GGC·GGT·GGT·ACC·AGC·CGC·CGG·CC

✓ 1µl (1γ) DP-L103
✓ 1µl (1γ) DP-L104
✓ 2µl 10X Ligase buffer (10x = .5M Tris/100mM DTT/150mM ...
✓ 0.5µl 5M NaCl [NaCl] = 100mM
✓ 20.5µl TE
-- 65°C 15', remove to 500ml 65°C bath, let cool
-- 4 to -20°C o/N.

Run 2λ pSJ36N⁻ SalI on 1% Agarose/TBE: See gel P34.
-- digest looks OK. ppt.

9 July 1991 Spin SJ36N⁻ SalI, dry, resuspend in 20λ H2O, cut c̄ Hin...
✓ 20λ DNA
✓ 5λ BRL2
✓ 24λ H2O
✓ 1λ HindIII (50U)
-- 37°C @ 8⁰⁰ AM, out at 11³⁰ AM.

To Page

Witnessed & Understood by me, | Date | Invented by / Recorded by DS [signature] | Date 7/9/91

Project No._____

Book No. 7 TITLE pSJ36NL

Page No.__ ppt, dry, to -20°C o/N.

July 1991

Resuspend Sal/HindIII digest in 25ul TE, run 2λ on 1% agarose/TBE:

-- can see very faint band (no picture)

-- set up ligation:

- ✓ 5ul SJ36N⁻ Sal/H3
- ✓ 2ul L103/L104
- ✓ 2ul 10X ligase
- ✓ 1ul 50mM ATP
- ✓ 9ul H_2O
- ✓ 1ul ligase

-- RT incubation 7:30 – 2:30

- add .5ul ATP/1ul ligase, RT to 3

Xform 10ul into HB101

Cut more pSJ36N⁻: 25ul DNA
10ul 10X BRL10
60ul H_2O
5ul (50U) SalI
} 37°C @ 3:45, out at 4:40 PM

-- 2ul to 1% agarose/TAE:

Cut OK.

ppt, spin, dry, resuspend in 20.5λ H_2O.

Cut with HindIII: 20.5λ DNA
2.5λ BRL2
2λ (40U) HindIII

-- 37°C o/N

July 1991

No colonies on transformation plates.

ppt. SalI/HindIII digest, spin, dry, resuspend in 25λ TE

To Page No.__

nessed & Understood by me,	Date	Invented by	Date
		Recorded by	7/11/91

TITLE _____ Project No. _____ Book No. 87

From Page No. — Set up ligation / 10 µl SJ36N~A3/SA1
/ 1 µl L103/L104
/ 3 µl 10X ligase
/ 1 µl 50mM ATP
/ 14 µl H_2O
- / 1 µl ligase
- - 16°C @ 11:25 AM

12 July 1991
Xform 12 µl into HB101; plate on 3 plates.

13 July 1991
No colonies on transformation plates

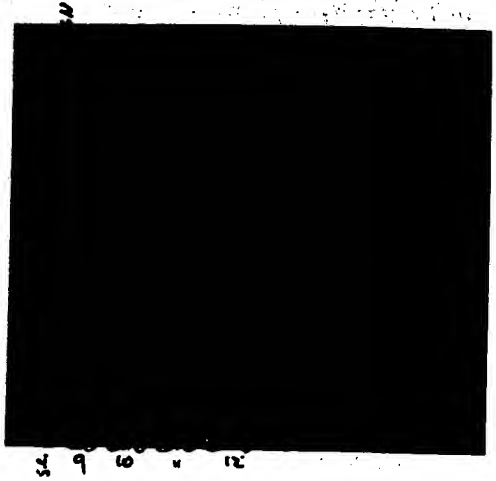
16 July 1991
Xform 5 µl of ligation mix into 13λ BRL ME-DH10 cells; on 2 plates

17 July 1991
plates OK : ~ 40 colonies on each. Pick 12 colo
to LB-Amp.

18 July 1991
Alkaline lysis preps on all 12; to -20°C O/N.

19 July 1991
QOH/$CHCl_3$ x1, $CHCl_3$ x1, ppt; resuspend in 50 µl TE

Cut/Σ Nco I: / 5 µl H_2O
/ 3 λ DNA
/ 1 λ BRL3
/ 1 λ Nco I
- - 37°C 45'.
run on .8% TAE gel:



David S. Pfaff 7/19/91

Witnessed & Understood by me,	Date	Invented
		Recorded

Project No.

Book No. 7 TITLE pSJ36NL

n Page No.

#1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12 all cut ε NcoI and ∴ have the link
#4, 10 don't cut ∴ toss these two.

[redacted]

Cut 10λ #1 ε NcoI: 10λ DNA
5λ BRL 3
33λ H_2O
2λ NcoI
∴ 37°C 1.5 hs; run 2 lanes, 8% Agarose/TAE: digest OK; ppt o/N.

2 July 1991

Spin Nco digest, resuspend in 25 ul, self-ligate:
25λ DNA
3λ 10x ligase Buffer -- to eliminate the possibility
1λ 50mM ATP of multiple linkers.
1λ Ligase
∴ 16°C @ 9:50 AM

Cut pSJ36NL #1 ε NcoI, NheI, NotI, SalI (all in linker); run on 1% Agarose/TBE gel:

[redacted]

All 4 enzymes cut!

innoculate 200ml culture.

3 July 1991

Qiagen prep of DNA; resuspend in 200ml TE:

-- 470ng/λ --

To Page No.

nessed & Understood by me, | Date | Invented by DS | Dat 7/23/91
Record d by

Project No._____
Book No. 7 TITLE pSJ36NL7

Page No.__

pSJ36NL2

Add the 5' polylinker to the pSJ36NL plasmid.

July 1991

Cut SJ36NL ē BamHI: 15ul (2.35ug) pSJ36NL
✓ 3ul BRL3
✓ 21ul H_2O
✓ 1ul BamHI (10U)
--37°C @ 8:30 AM.

Run 1.5ul (118ng) on 1% Agarose/TBE:

Anneal L101/L102: ✓1ul (1X) DP-L101
✓1ul (1X) DP-L102
✓ 2ul 10X ligase Buffer
✓ 0.5ul 5M NaCl $[Na^+Cl^-]_{total}$ = 100mM
✓ 20.5ul TE
--65°C 15', remove in 400ml 65°C beaker, let cool to RT.

QO 1ul/CHCl3 Bam digest, CHCl3 x1, ppt, resuspend in 20ul TE.

Set up ligation: 8ul pSJ36NL Bam/CIAP
✓ 1ul L101/L102
✓ 2ul 10X ligase
✓ 1ul dTP
✓ 7ul H_2O
✓ 1ul Ligase
--16°C O/N

5 July 1991
ligation to -20°C O/N.

7 July 1991
xform 5ul into competent HB101; on 3 plates

8 July 1991
Plates to 4°C -- by C. Schmidt

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nessed & Understood by m , | Date | Invented by DC Rau | Date 7/31/91
Recorded by

TITLE ~~pSJ36AL~~ [pSVΔ2N DHFR] Project No. ______ Book No. 37

From Page No. P54

31 July 1991

Pick 12 colonies to 5 ml LB-Amp; 37°C @ 9^00 /M

-- 2^15 PM: mini alkaline preps; resuspend DNA in 100λ

ΦOH/CHCl₃, CHCl₃, ppt, resuspend in 50λ TE.

1 August 1991.

Cut 3μl each c NcoI, PvuI, and NcoI + PvuI; run o
.8% agarose/TAE: load: Supercoiled
Nco I
PvuI
NcoI/PvuI

pSV2ΔDHFR NcoI = ~4000bp, 800bp (sites at ~700, 4700)
NcoI/PvuI = 2154bp, 1846bp, 800bp (PvuI at ~2854)

NcoI deletion: in SV40 ~2954   in DHFR ~2641
~1846   2154

#1, 2, 4, 6 have the NcoI deletion in the DHFR regi

#3, 5 have the NcoI del in the SV40 early region.

Cut 20λ #3 c NcoI; 50λ d [redacted]

-- 3λ to .8% agarose/TAE:

ppt. O/N.

Witnessed & Understood by me, | Date | Invented by J.S. R[illegible] Recorded by | Date 8/1/91

Project No.
Book No. 7 TITLE pSJ36NL ?

Page No. 50

31 July 1991

pick 12 colonies to 5ml LB-Amp @ 8^oo AM; 2^45 PM -- mini prep
QOH/CHCl3; CHCl3, ppt, resuspend in 50 λ TE.

August 1991

Cut 3 λ #1, 2, 3, 4, 5, 6 with Bam and Xba I; to .8% Agarose/TAE:
-- load S.K., Bam, Xba.

Linker
(in CAPS) →

g GATCCGGTCCGTCTAgatcc
c ctagGCCAGGCAGATCTAGg

Bam HI Rsr II XbaI

[redacted]

all cut enough Bam, not with XbaI -- toss.

Cut 7-12 w Bam and XbaI, screen .8% Agarose/TAE as above:

[redacted]

Again, none have the linker
-- toss all.

August 1991

Phosphatase SJ36NL Bam HI: 12 λ DNA
5 λ H2O
2 λ 10x CIAP Buffer
1 λ CIAP
-- 37°C 30', add 1 λ CIAP -- 37°C 30'
QOH/CHCl3 x1, CHCl3 x2, ppt.

To Page No. 2

n ssed & Und rstood by me, | Date | Inv nted by | Recorded by | Dat

TITLE pSVA2N⁻DHFR

Project No.

Book No. 87

From Page No. pSVA2N⁻DHFR

Want to knockout the NcoI site in the SV40 early promoter region.

24 July 1991

Cut pSVA2DHFR c NcoI: 12 ml DNA (7-18)
5 ml BRL #4
32 ml H_2O
0.5 ml (2.5U) NcoI
~37°C @ 11:10 AM; out @ 11:35 AM

Run on .8% Agarose/TAE prep gel:

good partial digest! Cut out linear band, gene clean, elute in 30 ml TE.

[redacted]

T4 Fill in ends: 130 ml DNA
✓ 4 ml 10X T4 Buffer
✓ 4 ml 10X dNTP's
✓ 1 ml H_2O
✓ 1 ml T4 polymerase
37°C @ 3:40 PM, out at 4:10 PM

DOH/CHCl3 x1, CHCl3 x1, ppt, resuspend in 20 λ TE, self-lig

✓ 5 ml DNA
✓ 2 ml 10X ligase
✓ 1 ml 50mM ATP
✓ 11 ml H_2O
✓ 1 ml ligase
~16°C o/N

25 July 1991

to -20°C.

To Page

Witnessed & Understood by me, | Date | Invented by | Date

Recorded by | 7/29/91

Project No.
Book No. E 2 TITLE pSVA2N-D"-R

Page No.

29 July 1991
Xform 5 µl into competent HB101; on 3 plates;

30 July 1991
Plates to 4°C by C. Schmidt.

31 July 1991
Pick 6 colonies to LB-Amp; to 37°C @ 9:00 AM.
2:00 PM: mini alkaline preps; resuspend DNA in 100 µl TE.
Do Ø/CHCl3 x1, CHCl3 x1, ppt, resuspend in 50 µl TE.

1 August 1991
SEE P. 51 for 8/1/91 Work -- OOPS!

2 August 1991
Spin NcoI digest, dry, resuspend in 19 µl H2O; T4 filling:
19 µl DNA
2.5 µl 10x T4 Buffer
2.5 µl 10x dNTPs
1 µl T4 Polymerase
-- 37°C @ 9:00 AM to 9:40 AM

Do Ø/CHCl3, CHCl3, ppt. Resuspend in 20 µl H2O, 1 µl to 8% Agarose

Looks good. to -20°C O/N.

To Page N

...ess d & Understood by me,	Date	Invented by D. S. Ø	Date 8/2/91
		Recorded by	

David S. Pfarr Notebook References for Humanized IgG Expression

1308F

Notebook	Procedure	Pages	Date
87	Immunoprecipitation of COS-1 produced H1308F (pSJ60/pSJ61)	65-66	7-31-91
95	Radiolabeling RSV F protein for H1308F immunoprecipitatons	4-5	7-29-91
95	CHO pSJ66-4 cell line passage for C. Schmidt	33-34	12-6-91
95	H1308F expressed from Merck Sharp and Dohme vectors in 293 cells	36-37	1-6-92
95	H1308F:R-S transfection in COS-1 cells	38-46	1-23-92
95	H1308F:R-S71 transfection in COS-1 cells	53-56	2-10-92
95	H1308F:66-71 transfection in COS-1 cells	61-65	2-27-92
95	1308F quantitation from COS-1 pSJ66 and COS pSJ60/pSJ61	100-102	9-10-91
95	H1308F purification from COS-1	103-106	10-2-91
95	H1308F purification from COS-1	107-108	10-4-91
95	H1308F purification from COS-1	110-111	10-7-91
95	H1308F purification from COS-1 pSJ66 and COS-1 pSJ60/pSJ61	115-117	10-9-91
95	H1308F purification from COS-1	119-122	10-18-91
95	H1308F purification from COS pSJ66 and COS pSJ60/pSJ61	123-127	10-22-91
95	H1308F purification from COS pSJ66	129-131	11-6-91
95	H1308F purification from CHO pSJ66	133-149	1-10-92
95	H1308F:R-S71 purification from COS-1	150-152	3-4-92
95	Western Blot: H1308F, H1308F:66-71, H1308F:S71	156-157	4-7-92
95	H1308F COS-1 supernatant analysis	171-172	9-12-91
95	H1308F COS-1 supernatant analysis	173-175	9-16-91
95	H1308F COS-1 supernatant analysis	176	9-20-91
95	H1308F COS-1 supernatant analysis	177	9-30-91
95	ELISA: purified H1308F 8-9-91	178	10-7-91

Exhibit 4

Exhibit 4

LE 1308F/rVacFr . IP #1 Project No. Bo No. 87

om Page No. 1308F/rVacFr RIP #1

C. Schmidt infected COS cells ± SJ60/SJ61 (1308F Human ?? H
and I infected BSC1 ± BSV-F vaccinia (Book 95, p 4-5). We
want to see if the 1308F is expressed in COS, and
if it is we want to know if it binds to BSV-F prote

31 July 1991

① Set up IP's (in microfuge tubes):

	Culture Supe (.4 ml)	Vaccinia Lysate
SJ60/SJ61 #1	.4 ml	.2 ml F / .15 ml TK⁻
SJ60/SJ61 #2	.4 ml	.2 ml F / .15 ml TK⁻
Mock #1	.4 ml	.2 ml F / .15 ml TK⁻
Mock #2	.4 ml	.2 ml F / .15 ml TK⁻
-- rotate @ 4°C : 1 hr -		
1308F (1 mg/ml) #1	1 µl / 400 µl PBS	.2 ml F / .15 ml TK⁻ } 4°C @ 2 hr
1308F (1 mg/ml) #2	1 µl / 400 µl PBS	.2 ml F / .15 ml TK⁻ }

② 4° : Add 100 µl Activated Staph A to each tube, on ice 10'
-- only enough Staph A cells for 11 samples; toss 1308F/TK

- Spin 5K, 5', aspirate
- resuspend in 200 µl IM Buffer, vortex, triturate
- Spin 5K, 5', aspirate
- resuspend in 200 µl RIPA
- Spin 5K, 5', aspirate
- Resuspend in 200 µl RIPA, transfer to new tube.
- Spin 5K, 5', aspirate
- to -20°C o/n

To Page N

Witnessed & Understood by me, Date

Invented by S.R. Date 7/31/91

Project Nr
Book No. 87 TITLE 1308F/rVacFr RIP #1

om Page No.__

1 August 1991

Resuspend pellets in 50 µl SDS loading dye; boil 7'

load 25 µl each onto 4-20% Protean II gradient gels:

Gel #1		Gel #2	
Lane	Sample	Lane	Sample
1	Rainbow Samples	1	—
2	Mock 1 - F	2	Rainbow Standards
3	Mock 1 - TK⁻	3	Mock 2 - F
4	SJ60/61 - F	4	Mock 2 - TK⁻
5	SJ60/61 - TK⁻	5	SJ60/61 2 - F
6	1308F - F	6	SJ60/61 2 - TK⁻
7	1308F - TK⁻	7	1308F 2 - F
8	—	8	—
9	—	9	—
10	—	10	—

-- dry gels, on film @ 4:00 PM.

1 August 1991

Develop film. Can barely see F protein in 1308F control lane, not in other lanes.

New film on @ 2:00 PM.

1 August 1991

Develop film, 9:00 AM -- Can definitely see F protein gel down by 1308F control (10 µg) and in the SJ60/61 lanes, but better in gel #2 than gel #1

To Page No.__

Witnessed & Understood by me, | Date | Invented by | Dat

Project No.
Book No. 5 TITLE rVAC-Fr

m Page No.

rVacFr Labeling

Want [35S] Met labeled RSV F protein to use in RIP ass. of 1308F antibodies.

9 July 1991

Rec'd. 4 plates of BSC1 cells from C. Schmidt, split 1:20 on 7/26/91.

① Thawed 2 virus stocks: rVacFr 1.7x10^10 PFU/ml
vSH.CS #4 2x10^10 PFU/ml -- TK^- vaccinia expressing malaria CS protein.

-- Sonicate 3 x 10 sec, in cup cicr, setting 8.

② Cells should be ~ 8·10^6/plate; want an MOI of 10 with the inoculum in 1.5ml medium (EMEM/2.5%FBS/L-Glu/NaPyr/P/S)

-- rVacFr: 8ml/3ml, for 2 plates
-- vSH.CS #4: 8ml/3ml, for 2 plates

Aspirate medium from plates, add 1.5ml inoculum, to 37°C.

③ Rock plates every 15' for 1.5 hr. -- 10^00 to 11^30

④ Add 8.5ml medium, to 37°C, for 2.5 hr: 11^30 - 2^00

-- 3 plates (one rVacFr and both TK^-) were too sparsely populated -- they were seeded lightly (1:20) on 7/26 for use later this week.

-- Toss these light plates, one rVacFr allowed to continue

-- Repeat steps 1-4 with 3 new plates, one rVacFr, 2 vSH.CS #4

⑤ inoculum on at 2^45 PM.
⑥ add 8.5ml medium @ 4^15 PM.

make medium: 5.44ml DMEM w/o Met
.056ml each: L-Glu, NaPyr, P/S
1.4ml DMEM complete + 10% FBS
7ml

T Page No.

tnessed & Understood by me, | Date 1/12/92 | Invented by | Date 7/26/91

TITLE rVacFr #1 — Project No. — Book No. 95

From Page No.

(c) 5:45 PM: Scrape cells off plates, transfer to 50ml tubes (combine rVacFr cells); spin 2K, 5'.

(D) aspirate medium, resuspend in 5ml DMEM w/o Met, spin.

(E) aspirate medium, resuspend:

① vSH.CS #4 in 1.5ml labeling medium w/o [35S] Met -- TK⁻ cold

② vSH.CS #4 in 1.5ml labeling medium, add 45ml [35S] Met @ 10.6 mCi/ml = 477 mCi

③ resuspend rVacFr in 3ml labeling medium, add 90ml [35S] Met = 954 mCi

-- Note: [35S] has already gone through 1 hr and only adding ½ as much label as ns

(f) to 37°C o/N.

30 July 1991 -- by Tom Furest:

Spin cultures, aspirate supes, resuspend in RIPA

① rVacFr: 2ml RIPA

② vSH.CS #4 TK⁻ Hot: 1ml RIPA

③ vSH.CS #4 TK⁻ Cold: 0.5ml RIPA

Vortex vigorously, to -20°C.

31 July 1991

Thaw lysates, spin 30K, 30min, 4°C SW50.1 rotor

Remove supes to 3ml tubes; cleared lysates.

To Page

Witnessed & Understood by me, — Date — Invented by — Date

TITLE SJ66-4 10^{-8}M MTX and 10^{-7}M MTX Maintenance Project No. 98 book No. 95

From Page No. SJ66-4 10^{-8}M MTX and 10^{-7}M MTX

6 December 1991

Harvest cells, resuspend in 10ml, count:

SJ66-4 10^{-8}M MTX: 4.5×10^5 cells/ml x 10ml

-- Seed 1 flask @ 4×10^5 cells/ml x 10ml = (P1

SJ66-4 10^{-7}M MTX: 6.0×10^5/ml x 10ml

-- Seed 1 flask @ 4×10^5/ml x 10ml = (P8)

9 December 1991

T75's look stressed -- cells withdrawing and many flo

Harvest cells, count:

SJ66-4 10^{-8}M MTX

6.0×10^5 cells/ml x 10ml, 100% viable

-- Seed 1 T75 @ 2×10^5/ml x 10ml = (P11)

SJ66-4 10^{-7}M MTX

5.5×10^5 cells/ml x 10ml, 87% viable

-- Seed 1 T75 @ 2×10^5/ml x 10ml = (P9)

-- Seed remaining cells into a T150 in 35ml medium

13 December 1991

Harvest T75's, count:

~~Dead~~

SJ66-4 10^{-8}M MTX : 5.7×10^5/ml

SJ66-4 10^{-7}M MTX : 9.2×10^5/ml

Harvest T150s seeded 12/9, pool cells with T75 cells, Count:

To Pag

Witnessed & Understood by me, Date 12/13/91 Invented by [signature] Date 12/13/91

Project No. 40
Book No 5 TITLE SJ66-4 10^{-8} and 10^{-7} MTX

m Page No.

SJ66-4 10^{-8} MTX: 2.54×10^6 cells/ml × 13ml

SJ66-4 10^{-7} MTX: 2.71×10^6 cells/ml × 13ml

-- Seed 1 spinner each @ $2 \cdot 10^5$/ml × 100ml

December 1991

SJ66-4 10^{-8} M MTX spinner contaminated ~ bacteria; TOSS

Count SJ66-4 10^{-7} M MTX: 7.8×10^5/ml, 91% viable -- many clumps of cells in "strings" that could'nt be counted.

Spin 50ml, resuspend in 25ml, count: 9.2×10^5/ml

-- Seed ~~#~~ 1 spinner @ 2×10^5/ml × 100ml

December 1991

Cell count: 6.6×10^5/ml, 53% viable

Spin 70ml, resuspend in 25ml: 1.26×10^6/ml, 60% viable

Seed 19ml → 170ml spinner: 2×10^5/ml × 120ml

Obviously leaving cells in this medium for > 3 days is quite detrimental to their health. Until some medium development gets done I won't be able to grow cultures productively for more than 72 hrs.

3 December 1991

Cell count: 4.5×10^5/ml, 85% viable Cells quite clumped and aggregated! Other cell lines are coming along that are much better.

TOSS cells.

T Page No.

nessed & Understood by me, | Date 8/5/92 | Invented by ~~Dan S. Kaufman~~ | Date 2/23/91

Project No.: 03.95
Book No. 15 TITLE 293 Transfection 447D : 1308F

Page No.

293 Transfection
1308F and 447D Cotransfections

I want to compare the productivity of the Mark vector/293 transient system versus the SV40 ori/COS system to see which gives higher yields of protein. Colleen Schmidt will do the COS transfection in parallel with my 293 transfect

January 1997

Harvest 293 cells, resuspend in 20ml (LNB95-23); count:

1.72 x 10^6 cells/ml

Seed 7 plates ≈ 2.3ml cells (4 x 10^6 cells/plate) in 12ml total.

January 1997

Ppt. DNA for xfections:

1308F

PM1-1308FH4 @ 0.57 ug/ml - 70ml = 40mg } Combine and ppt
PM3-1308FL6 @ 0.49 ug/ml - 82ml = 40mg } o/n.

447D:

H1447D @ 1.03 mg/ml - 37ml = 40mg } combine and ppt
L3447D @ 0.725 mg/ml - 55ml = 40mg } o/n.

January 1997

Spin DNAs, 70% wash, air dry in hood.

① Resuspend DNAs in 1ml TBS each
② Add DNA/TBS to 4ml DEAE-Dextran/Chloroquine/DMEM
③ Wash 6-Pl00's ≈ PBS, add 2.5ml DMEM/10% NuSerum per plate.
④ add 2.5ml DNA/DEAE-Dextran to each of 2 plates for 1308F, 447D, /
⑤ 6 Hrs later - DMSO shock cells (10% Dmso/PBS)
-- cells lifting off plate in Dmso shock, lost ~ 30%-50% of cells.
⑥ Add 15ml medium to each plate.

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TITLE 293 Transfection: 447D + 1308F

Project No. 83.95
Book No. 95

From Page No.

10 January 1992

4^oo PM: Harvest medium from plates, spin, decant, resusp cell pellets in 15ml medium :- return to plates.

- Save 1ml aliquot from each plate for assay, pool remain media, store all @ -20°C.

13 January 1992

4^oo PM (72 hrs) Harvest medium, separate samples from each plate for assay, pool like medium. To -20°C.

Toss plates -- cells ~90% sloughed off.

14 January 1992

ELISA: No Ab seen in any of the samples in GαHIgG ELISA.

B7 dem ELISA: No Ab detected binding to the B7 protein.

--The DEAE-Dextran transfection of the 293 cells obv did not work, since parallel transfections into Cos Cells (C1S) did work. Will either have to work out the conditions for DEAE-Dextran mediated transfection of 293 ce or use another transfection method.

To Page N

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Project No.. 98
Book No.. 5 TITLE COS: H1308F R→S, H1129h/l, Ch1129h/l

m Page No.__

COS Transfection

H1308Fh: R→S / H1308Fl: H1308F R→S mutation
CH1129h/Ch1129l: Chimeric 1129
H1129h/H1129l: Humanized 1129

3 January 1992

Coprecipitate DNAs for 2-60mm dishes for each transfection:

H1308Fl/H1308Fh: R→S
SJ61: 1.72 mg/ml : 11.6 ml
R→S #1: 0.67 mg/ml : 30 ml
R→S #2 = 0.36 mg/ml : 56 ml

Ch1129h/Ch1129l
pSJ75 = 0.82 mg/ml : 24 ml
Ch1129l #1 = 0.25 mg/ml : 80 ml
Ch1129l #2 = 1 mg/ml : 20 ml

H1129h/H1129l
H1129h = 5.34 mg/ml : 3.7 ml
H1129l #1 = 0.32 mg/ml : 63 ml
H1129l #2 = 0.4 mg/ml : 50 ml

Humanized and chimeric light chain transfections are mislabeled. Save these for later CH/H mixing experiments!

4 January 1992

Coprecipitate more DNAs:

CH1129h/CH1129l
pSJ75 = 0.82 mg/ml : 24 ml
CH1129l #1 = 0.32 mg/ml : 63 ml
CH1129l #2 = 0.4 mg/ml : 50 ml

H1129H/H1129l
H1129h = 5.34 mg/ml
H1129l #1 = 0.25 mg/ml : 80 ml
H1129l #2 = 1 mg/ml : 20 ml

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	1/17/92		1/7/1

Project No .98
Book No 95 TITLE COS: H1308f h:. i; H1129 h/l; CH1129 l/e

From Page No.__

Cleanup Samples for Western blot using Staph-A cells (Pansorbin cells):

(1) Activate 1ml pansorbin cells:
-- Spin, decant
-- Resuspend in 1ml STE-.5%NP40, RT 20'
-- Spin in microfuge, setting 5, 5'; decant
-- resuspend in 1ml STE-.05%NP40, RT 5'
-- Spin, decant.
-- Resuspend in 8ml STE-.05%NP40 + 1mg/ml Ovalbumin, store on ice.

(2) Bind Mabs to Staph A:
-- add 1ml of each combined supernatant to .1ml Activated Pansorbin cells; add 1ml SJ66 Fucia #2 GXH Flow Thru #2 (1.N095-149: 1.87mg/ml) to .1ml cells.
-- Rotate, 4°C, 15'; on ice 10'

(3) Wash cells:
-- pellet cells, setting 5 (Biofuge), 5', decant
-- resuspend in .5ml 1M Buffer
-- pellet, decant
-- resuspend in 1ml RIPA
-- pellet, decant
-- resuspend in .5ml RIPA, transfer to clean tube
-- pellet, decant, to -20°C O/N.

29 January 1992

Resuspend pellets in 100µl 1X loading buffer, boil 5', Spin 5' in microfuge, remove supes. to new tube.

Load 25µl of each sample onto 15% Disc-SDS/PAGE

Lane	Sample
1	Amersham Rainbow stds.
2	SJ66 Fucia #2 Flow Thru #2
3	Mock
4	H1308F: R-75 #1
5	H1308F: R-75 #2
6	CH 1129 #1
7	CH 1129 #2
8	H1129 #1
9	H1129 #2

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Witnessed & Understood by me, [signature] Date 1/1.

Invented by [signature] Date 1/29/92

11

TITLE COS: H1308F R-S; H1129 1/2; CH1129 1/2 — Project No. 98 — book No. 95

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- Run dye front to ~ 1cm from bottom.
- Blot to .2 μm nitrocellulose: 100V, 1 hr.
- PBS wash blot, dry o/N.

30 January 1992

- Rocky brake blot in H_2O; block in TBS-5% milk 1 hr, RT.
- Wash 15' PBS-Tween, wash 2x 5' PBS-Tween
- Add 10 ml GαH IgG-HRP (1:1000), incubate RT 1 hr.
- washes above, all but ECL reagent, incubate 1 min, expose to film.

Results

The IgGs are all unbroken and running at ~160 kd!! Perhaps I ... too long before loading the gel. A BSA band can be seen in all the lanes that have TC supernatant samples, so the IP didn't get rid of ... all of the BSA.

Repeat gel -- boil samples

-- 4 samples popped open and flooded out during last boil: both H... One mock and one CH1129. Toss all samples and start over.

No Pansorbin cells left, so will use Protein A-Sepharose to immunoprecipitate samples.

① Add 1.28 mg of each Ab supernatant to a microfuge tube

Mock - 1 ml
H1308F R-S #1 - .75 ml
H1308F R-S #2 - .57 ml
CH1129 #1 - .9 ml
CH1129 #2 - 1 ml
H1129 #1 - 1 ml
H1129 #2 - 1 ml SJ66 Fucia #2: Clowther #2 - .68 ml

-- bring up to 1 ml ≈ DMEM/10% NS

② Add 150 ml Protein A-Sepharose to each tube, rotate 4°C, 1 hr + 15'.
③ Spin 1' microfuge, decant, resuspend in 1 ml PBS - .5M NaCl - 1% Triton, spin, d...
④ Repeat above wash; wash 2x in PBS-1% Triton and 2x in PBS.
⑤ Resuspend final pellet in 100 ml 1.2x Sample loading buffer. To -2C

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Witnessed & Understood by me, | Date 1/1 | Invented by [signature] | Date 1/31/92

Project No. 98
Book No. 5 TITLE COS: H1308F R→S, CH1129; H1129

Page No.

January 1992

Boil Samples 5', spin 30" microfuge
Load 20 µl onto 15% SDS-PAGE

Lane	Sample
1	~~Rainbow Stds~~ empty
2	Rainbow Stds
3	SJ86 Fwin #2 FlowThru #2
4	Mock
5	H1308F R→S #1
6	H1308F R→S #2
7	CH1129 #1
8	CH1129 #2
9	H 1129 #1
10	H1129 #2

Run gel, blot to nitrocellulose, wash in H_2O, dry, to 4°C O/N.

All flasks of BSC cells from SJ (T25's):
① infected ≥ Vac F virus, ^{35}S labeled
② infected ≥ TK⁻ virus, ^{35}S labeled
③ uninfected, ^{35}S labeled

-- Harvest cells, PBS wash, resuspend in 1ml RIPA, Spin 30k, 30', SW55T,
-- Remove Supes to clean tubes, -20°C O/N

February 1992

Rehydrate Blot, block in 5% Milk-TBS RT, 1hr.
Probe ≥ GαHIgG-HRP, 1:1000, RT 1hr.
Wash, ECL detect, expose to film

Results

H1308F:R→S #1 and #2, and CH1129 #1 and #2 ~~bt~~ DSP all show good amounts of Ig -- both heavy and light chain without a gross excess of light chain. Neither H1129 shows any Ig -- heavy or light chain, corroborating the results seen in the ELISA.

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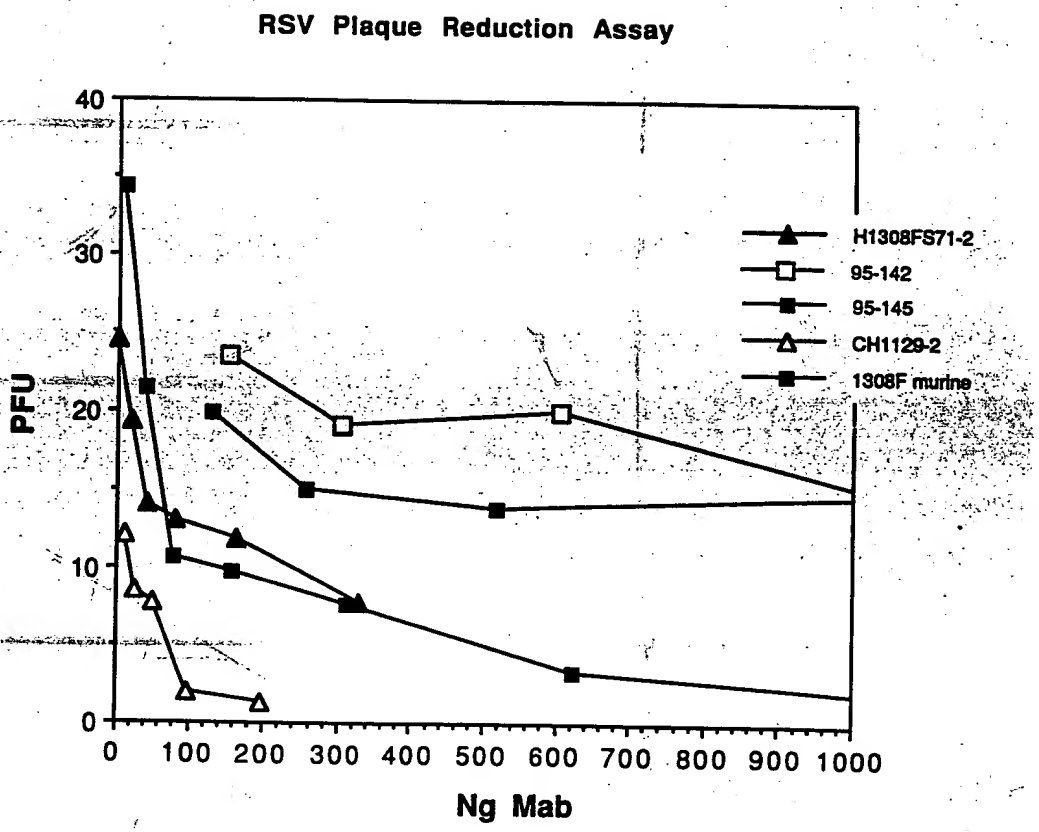
essed & Understood by me,	Date	Invented by	Date
	2/17/97		2/3/97


RSV Plaque Reduction Assay
PFU
Ng Mab
0
10
20
30
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100
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300
400
500
600
700
800
900
1000
H1308FS71-2
95-142
95-145
CH1129-2
1308F murine

TITLE COS: H1308F R→S; CH1129; H1129

Project No. 98
Book No. 95

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Note: transfection plates were harvested again on 1/30/92; 2k cells were also h and the pellets frozen away.

ELISA Results	1/24 → 1/27	1/27 → 1/30	
H1308F:R→S #1a	1.66 ng/ml	6.30 ng/ml	
H1308F:R→S #1b	1.75 ng/ml	4.32 ng/ml	
H1308F:R→S #2a	2.63 ng/ml	7.62 ng/ml	Combined sample reassayed 2/10/92: 5.41 ng/ml
H1308F:R→S #2b	1.86 ng/ml	5.50 ng/ml	
CH1129 #1a	1.23 ng/ml	5.84 ng/ml	Samples are all ~4.5ml each.
CH1129 #1b	1.59 ng/ml	5.34 ng/ml	
CH1129 #2a	1.34 ng/ml	4.45 ng/ml	Combined sample assayed again 2/10/92: 3.27 ng/ml
CH1129 #2b	1.22 ng/ml	3.43 ng/ml	
H1129 1a	-0- ng/ml	ND	
H1129 1b	-0- ng/ml	ND	
H1129 2a	-0- ng/ml	ND	
H1129 2b	-0- ng/ml	ND	
Mock 1	-0- ng/ml	-0- ng/ml	
Mock 2	-0- ng/ml	0.17 ng/ml	

RIP

Need to see that both H1308F:R→S and both CH1129's still bind to the F protein of

① Preadsorb Vac lysates
-- add .5ml 1/24-1/27 Mock COS supe to each lysate (1/24/92), rotate 1.5 hrs @ 4°C.
-- add 100µl Pansorbin cells to lysates, rotate 4°C 30', spin, remove supes to new t

② Bind Supes (1/24-1/27) to lysates
-- Add 2ml lysate to .5ml COS-supes from 1/24-1/27; rotate 1hr. @ 4°C.

③ ~~Vac~~ Adsorb to Staph A
-- add 100µl Pansorbin cells to each supe, rotate 1hr @ 4°C.
-- spin 5' @ 5 on Biofuge A, decant
-- wash x .5ml 1M Buffer, spin, decant, repeat spin wash
-- wash x .5ml RIPA, spin, decant, repeat wash; transfer to fresh tube, spin, dec
-- Resuspend in 100µl 1.2X Sample loading buffer.

④ load 25µl onto 15% SDS-PAGE
-- boil samples 5'
-- spin 12k, biofuge, 5'

To Page

Witnessed & Understood by me, | Date | Invented by | Date 2/6/92

Project No. 98
Book No. 5 TITLE COS: H1308FR-s; C. 29; H1129

n Page No.

Gel #1

Lane 1 - rainbow markers
2 - Mock/F lysate
3 - Mock/TK⁻ lysate
4 - Mock/uninfected lysate
5 - H1308F #1/F lysate
6 - H1308F #1/TK⁻ lysate
7 - H1308F #1/uninfected lysate
8 - H1308F #2/F lysate
9 - H1308F #2/TK⁻ lysate
10 - empty

Gel #2

Lane 1 - rainbow markers
2 - Mock/F lysate
3 - Mock/TK⁻ lysate
4 - Mock/uninfected lysate
5 - CH1129 #1/F lysate
6 - CH1129 #1/TK⁻ lysate
7 - CH1129 #1/uninfected lysate
8 - CH1129 #2/F lysate
9 - CH1129 #2/TK⁻ lysate
10 - empty

Run gels, soak 1 hr. in 1M Na Salicylate, dry, on film at 8³⁰ PM.

During washes, realized that I had activated the Panserbio cells! Repeat RI

1/14
① Spin 5ml Panserbio cells, resuspend in .5ml STE-.5%NP40, RT 15'.
② Spin cells, resuspend in .5ml STE-.05%NP40
③ Spin cells, resuspend in .5ml STE-.05%NP40 - 1mg/ml Ovalbumin
④ Bind Supers to lysates
...add 0.1ml lysate (TK⁻ and Vac F) to .25ml COS Supers, rotate 1hr @ 4°C.
⑤ Add 50µl Activated Panserbio cells to Supers/lysates; rotate 30' 4°C.
⑥ Wash cells as above (LNB95-43), use .2ml volumes
⑦ Store final pellets @ -20°C.

February 1992

Resuspend RIP #2 pellets in 50ml 1.2X Sample Buffer, boil 5', spin in ~~micro~~ microfuge 5', load 20ml on 15% SDS-PAGE:

Gel 1

Lane 1 - Blank
2 - [¹⁴C] Rainbow Markers
3 - Mock/F lysate
4 - Mock/TK⁻ lysate
5 - H1308F R-s #1/F lysate
6 - H1308F R-s #1/TK⁻ lysate
7 - H1308F R-s #2/F lysate
8 - H1308F R-s #2/TK⁻ lysate
9 - Blank

Gel 2

Lane 1 - [¹⁴C] Rainbow Markers
2 - Blank
3 - Mock/F lysate
4 - Mock/TK⁻ lysate
5 - CH1129 #1/F lysate
6 - CH1129 #1/TK⁻ lysate
7 - CH1129 #2/F lysate
8 - CH1129 #2/TK⁻ lysate
9 - Blank

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nessed & Understood by me, | Date 2/14/92 | Invented by [signature] | Date 2/14/92

Cos: H1308F R+s, CH1129; " 1129

--Pungrel, fix in destain 30', wash 5' in H_2O, soak in 1% NaSalicylate 30'
On grel @ 5:30 PM.

RIP #1: develop grel @ 7:30 AM (11 hr exposure): very faint; can see S bands in all infected lanes but not in uninfected lanes, so these are obviously vaccinia lanes. Can see F protein in both H1308F/VacF lanes and in both CH1129/VacF lanes. Both H1308F R+s and both CH1129 molecules bind to RSV-F protein.

--put new film on blot @ 7:30 AM.

5 February 1992

Develop RIP #2 film at 8:30 AM (15 hr exposure): very clean gel. Can see RSV-F bands in all 4 H1308F R+s and CH1129 lanes; very light exposure.

--put new film on gels at 8:30 AM

6 February 1992

Develop RIP #1 film: - 55 hr exposure

(1) There are ~7 major background bands that are attributable to the Cells/Vaccinia system.

(2) Can see both RSV-F bands only in the H1308F R+s and CH1129 / with the F lysates. All four constructs are producing functional

7 February 1992

Develop RIP #2 film, 55 hrs:

(1) Very clean -- can see RSV-F bands only in the H1308F R+s and lanes with VacF lysates; No other background bands are really visible. Apparently activating the Staph:A cells does not increase the capacity/ability to bind Igs, but it does drast reduce the background.

10 February 1992

2.5 ml each of: 1/30 Mock
1/30 CH1129 #2
1/30 H1308F S-71 #2

} given to F. Brady and J. Tamura

additional 2.5 ml to CHR 2/18/92 for Neut ass
DSP 2/18/92

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Project No. 98
Book No. 2 TITLE COS: H1308F-S71, H1129; H1129

n Page No.

2 February 1992
Ran RIP against ^{35}S-Fprotein (LNB95-49) using all the propper (avid
toad - controls. Very light exposure shows that CH1129 a2(430) pulls down
specifically the Fprotein.

3 February 1992
50 hr. exposure of RIP shows very clearly that the RSV-F protein is
immunoprecipitated with the CH1129.

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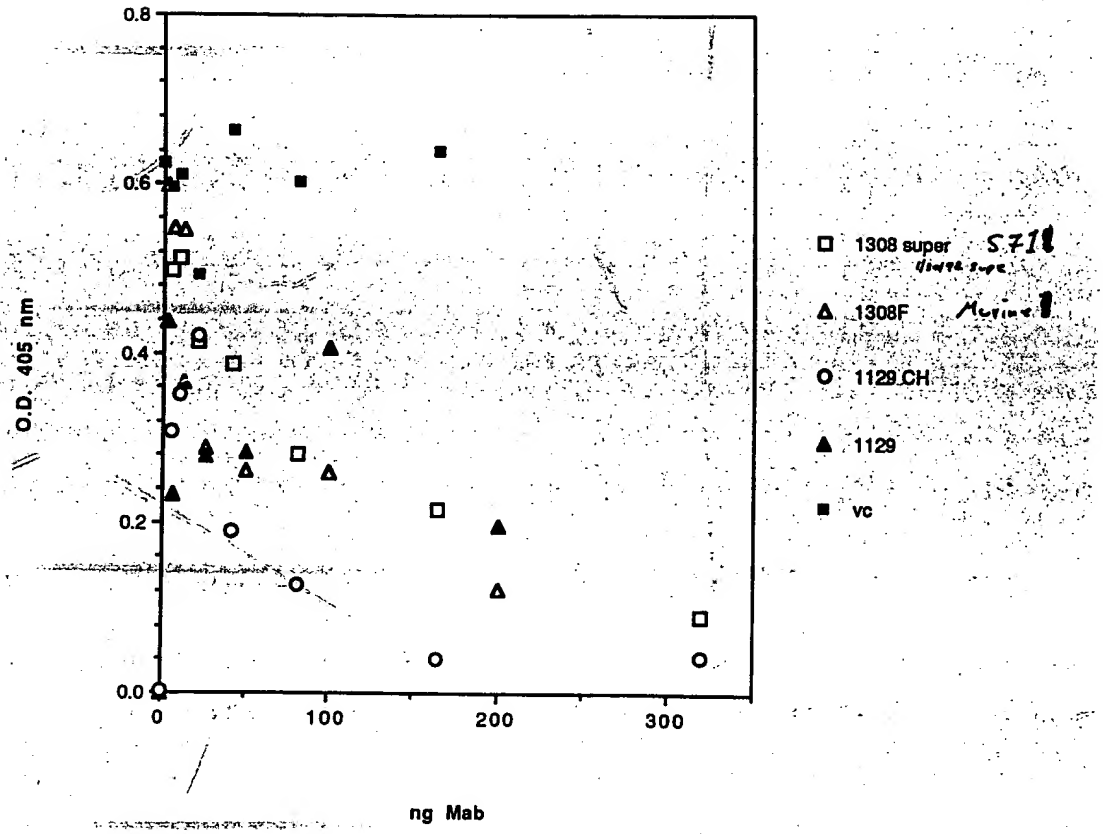
2/14/92

RSV Microneutralization



TITLE COS: H1308F-S71; CH1129; MEV98-6 H/L; H1129 h+l

Project No. 98/.83
Book No. 95

From Page No.

COS Transfection

H1308F-S71 and CH1129 and MEV98-6 H/L and H1129 #1 and #6

Want to produce more H1308F-S71 (formerly H1308F R+S #2) and CH1129 (#2) further analysis; want to see if MEV98-6H and MEV98-6L express their respective

10 February 1992

Harvest COS1 cells, count: 2×10⁶/ml ×20ml
Seed 10 P100s at 3·10⁶ cells/plate.

11 February 1992

Precipitate DNAs:

① H1308F·S71 #2
-- 46λ pSJ61 (80γ)
-- 111λ H1308F·S71 #2 prep 1 (40γ)
-- 63λ H1308F·S71 #2 prep 2 (40γ)
} ppt, 70% wash, resuspend in 2ml TBS

② CH1129 #2
-- 69λ pSJ75 (56γ)
-- 242λ CH1129l #2 prep 2 (62γ)
} ppt, 70% wash, dry, resusp in 2ml TBS

③ 98-6
-- MEV98-6 H · 80λ (20γ)
-- MEV98-6L · 20λ (20γ)
} ppt, 70% wash, dry, resuspend in 0.5ml TBS

DEAE/Dextran transfect as usual, 4 plates c̄ H1308F·S71, 3 p c̄ CH1129, 1 plate with 98-6, 1 plate c̄ H1129 H/l #1, 1 plate c̄ H1129 h/l #6.

-- H1129h: 3.7 μl (20γ) for each xfection.
-- H1129l #1 (2·11·92, SJ): 29 μl (20γ)
-- H1129l #6 (2·11·92, SJ): 25 μl (20γ)

-- DNA on cells 6.5 hrs. before DMSO shock.

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Project No. 98/.83
Book No. 15 TITLE COS: H1308F-S71; 1129; MEV98-6 hvl; H1129 hvl

m Page No.

4 February 1992

Harvest supes at 96° AM (63.5 hrs); ELISA:

Purified Lots 48	H1308F-S71: 3.11 ug/ml	x 38 ml
	CH1129: 4.72 ug/ml	x 28.5 ml
	H1129 #1: 102 ng/ml	x 9.5 ml
	H1129 #6: 248 ng/ml	
	MEV98-6: 3.98 ug/ml	

2.5 ml given to CHR 2/18/92 for Neut assays. DSL 2/14/92

Feed plates with 10 ml medium

-- The H1129s are over a log lower than everything else, there is a question of the accuracy of the light chains, so there may be bad constructions. Toss the #1129 #1 and #6 plates.

-- The MEV98-6 expresses, and at high levels!! The crippled SV40 early region still retains sufficient information to allow for the replication of the plasmids. Also, the 98-6 heavy and light chains apparently have no major frame shifts since intact Ab is being produced.

-- High levels of both CH1129 and H1308F-S71 are being produced.

7 February 1992

Harvest supes from plates; feed plates ε 10 ml medium.

Western: Same as western LNB95-48

-- Activate Staph A cells

-- Add 100 microliters to:

(A) 1.5 ml Cos Mock supernatant
(B) 1.5 ml H1129 #1 (2/14): 153 ng
(C) 1.5 ml H1129 #6 (2/14): 372 ng
(D) 0.5 ml MEV98-6 (2/14): 1.99 ug
(E) 1 ml 1.35 mg/ml HIgG1,κ diluted to 1 ml in PBS
(F) 1.25 ml H1308F 95-121 fraction #3 = 1.01 mg

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	2/17/92		2/17/92

TITLE COS: H1308F-S71; CH1129; 1-98-6 bnl; H1129 bnl

Project No. 98.83
Book No. 95

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-- rotate suspensions, 4°C, 45'
-- pellet, resuspend in .5ml 1M buffer, pellet
-- resuspend in .5ml RIPA, pellet
-- resuspend in .5ml RIPA, transfer to new tube, pellet
-- resuspend in 100µl 1X loading buffer.
-- to -20C O/N.

18 February 1992

Boil samples 5', spin 7' in microfuge, load 25µl on 4-20% Gradient SDS
-- See LNB95-48 for order and run time.

Western Results

-- 98-6 seems to be running slightly fast, both heavy and light chains. This needs to be repeated

-- H1129 bnl #6 can be seen very faintly, however 13 fold and respectively, less protein were present in these supes. sizes do match up with the H/CH 1129 bands.

ELISA of 2/17/92 supes:

H1308F-S71: 3.9 µg/ml
CH1129: 5.9 µg/ml
MEV98-6: 6.7 µg/ml

Repeats of 2/14 samples:
H1308F-S71: 2.44 µg/ml (3.11) x̄ =
CH1129: 4.17 µg/ml (4.72) x̄ =

20 February 1992

Harvest COS supes; ELISA:

H1308F-S71: 1.97 µg/ml
CH1129: 2.93 µg/ml
98-6: 6.74 µg/ml

Ran RIP against ^{35}S-F (LNB95-49): 14hr exposure shows that 98-6 does not ppt. the F protein. No F is seen in either H1129 ~~either~~ A longer exposure may be needed.

To Page

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Project No. 98/183
Book No. 15 TITLE COS: H1308F.s71; Lm1129; MEV98-6 hvl; H1129 hvl

1 February 1992

98-6 Western to ascertain binding to GP160.

Run 12|576 SDS-PAGE:

Lane	Sample
2	200ng gp160(IIIB)
3	100ng gp160 "
4	50ng gp160 "
5	25ng gp160 "
6	Rainbow Markers
7	25ng gp160 (IIIB)
8	50ng gp160 "
9	100ng gp160 "
10	200ng gp160 "

--gp160(IIIB) from S. Heim
- Blot to NC - 1hr|120V
- Block 30', RT, 5% M.Mk|PBS
- Cut blot into 3 strips
  ① lanes 1-5 + ½ lane 6
  ② ½ lane 6 + lanes 7-8
  ③ lanes 9-10

Incubate strip #1 with 2/17/92 98-6 supe (6.7mg/mL) diluted 1:2 in PBS-T
Incubate strip #2 with 447D (20 mg/mL) 1:1000 in PBS-T
Incubate strip #3 with Cos-Mock supe diluted 1:2 in PBS-T
-- RT 1hr.
--wash blots x PBS-T, place in same containers, and probe x GAM Ig-HRP,
- wash, detect x ECL:
① 447D, even in long (10') exposure does not detect GP160 IIIB; 447D was selected against MN strain and only reacts weakly with IIIB
② Mock control detects nothing.
③ 98-6 lights up GP160!! In 1' exposure can clearly see 200ng, 100ng, & 50ng gp160 with very little background. In very long exposures (5' and 10') the rainbow markers are detected as well as a few other background bands.

February 1992

RIP 50 hour exposure:
① 98-6 does not precipitate F protein of RSV
② H1129 #1 and H1129 #6 do not precipitate RSV-F. Since the H1129h chain does work (LMR05-5a) this bears out the data about the H1129l chains.

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TITLE COS: H1129h+l; CH/H1129; H1308F: H66-71

Project No. 98

Book No. 95

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COS Transfection

H1129 h+l - 1B, 6A, 7A

CH1129γ1 + H1129 l-6A

H1308F: H66-71

27 February 1992

Seed 8 P100's @ 2·10^6 cells/plate/10ml, P58

28 February 1992

ppt. DNAs:

H1308F: 66-71 (40γ ea) ~~23~~ 50μl h, 23μl l

H1129-1B: 19μl h, 36μl l

H1129-6A: 19μl h, 38μl l

H1129-7A: 19μl h, 53μl l

CH1129γ1/H1129l-6A: 23μl h, 38μl l

Spin, wash c̄ 100% EtOH, decant in hood, dry.

Note: Remaining xfection steps by CAS up to DMSO shock.

DNA on cells at 12⁰⁰; shock cells @ 6⁰⁰ PM; feed with 12ml 10% NS medium.

2 March 1992

Harvest sups; refeed plates c̄ 15ml 10% NS medium

ELISA

H1129-1B:	< 50ng/ml
H1129-6A:	77ng/ml
H1129-7A:	320ng/ml
CH/H1129-6A:	140ng/ml
H1308F: 66-71 #1:	< 50ng/ml
H1308F: 61-71 #2:	< 50ng/ml

Values are very low! Either there is a problem with the DNAs (bad cons, incorrect concentrations, not resuspended in TBS) or there was a problem with the transfect...

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Book No. 15 TITLE COS: H1129h+l; cH/H1129-6A; H1308F: H66-71

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3 March 1992

Set up 7 P100s @ $2 \cdot 10^6$ cells/plate/10ml, P60, for a repeat transfection including a positive control.

Precipitate DNAs:

H1129-1B: SJ81 (heavy chain) - 19 µl
light 1B - 36 µl

H1129-6A: SJ81 (heavy chain) - 19 µl
light 6A - 38 µl

H1129-7A: SJ81 (heavy chain) - 19 µl
light 7A - 53 µl

CH1129x/H1129/6A: SJ75 (heavy chain chimera) - 23 µl
light 6A - 38 µl

H1308F: H66-71: heavy - 25 µl
SJ61 (humanized light) - 11.6 µl

H1308F-S71: SJ82 (humanized heavy S-71) - 3 µl
SJ61 (humanized light) - 11.6 µl

Spin, decant, wash ± 100% EtOH, decant & dry in hood. -- to /-20°C o/n.

Dilute DNAs to be transfected to 100ng/λ in TE, run 200ng each on .8% Agarose/TAE gel to be certain that the DNAs are intact and the concentrations are reasonably accurate

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Project No. 98

TITLE COS: H1129hGL; CH/H1129-6A; H1308F:H66-71; H1308F:H71 Book No. 95

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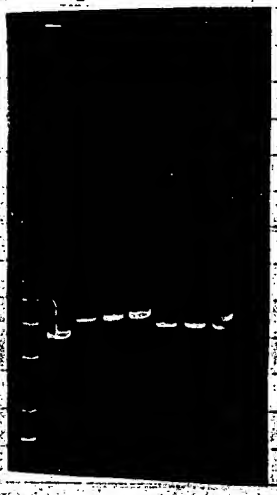

DNAs all look intact and all within 5fold of each other in concentration.

4 March 1992

Resuspend DNAs in 0.5ml TBS, add to 2.5ml DCAE-Portion of DMEM and transfect 1-plate each along with one mock p.

- DNA on cells at 9:00 AM
- Shock cells @ 5:00 PM = 8hrs; feed cells with 15m

6 March 1992

Harvest supes from first transfection

ELISA (6.3.92):

H1308F:66-71 #1:	<50ng/ml
H1308F:66-71 #2:	<50ng/ml
H1129-1b:	<50ng/ml
H1129-6A:	245ng/ml
H1129-7A:	925ng/ml
CH1129-6A/H1129-6A:	2.1mg/ml

Note: H1308F:66-71 did not expr at all in this transfection. H1129-1b did not express at all in this transfection.

Toss cultures.

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4 Project No. 98
Book No. 15 TITLE COS: H1129 hrl, H/H1129-61; H1308F:66-71

rom Page No.

7 March 1992

Harvest supes from 3/4 transfection; feed plates ε 15ml medium.

ELISA (3-9-92)

Mock:	< 50ng/ml
H1308F:S71:	1.4 ug/ml *
H1308F:66-71:	< 50ng/ml
H1129-16:	< 50ng/ml
H1129-61:	60ng/ml *
H1129-71:	< 50ng/ml
CH1129/H1129-61:	< 50ng/ml

2 March 1992

Harvest supes from 3/4 transfections (5days); feed plates ε 15ml.

ELISA

Mock:	< 50ng/ml
H1308F:S71:	2.17 ug/ml *
H1308F:66-71:	< 50ng/ml
H1129-16:	< 50ng/ml
H1129-61:	.99 ug/ml * -- To ST 3/18/92
H1129-71:	68 ng/ml *
CH1129/H1129-61:	1.99 ug/ml *

Still the H1308F:66-71 does not express, nor does the H1129-16. The H1129-16 construct does have some errors which may explain the lack of expression. There is no apparent reason for the H1308F:66-71 not to express.

13 March 1992

Toss transfection plates, cells are all pretty toasted!

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TLE COS: H1129 h.l. CH/H112 1, H1308F:66-71

Project No. 70

Bc No. 95

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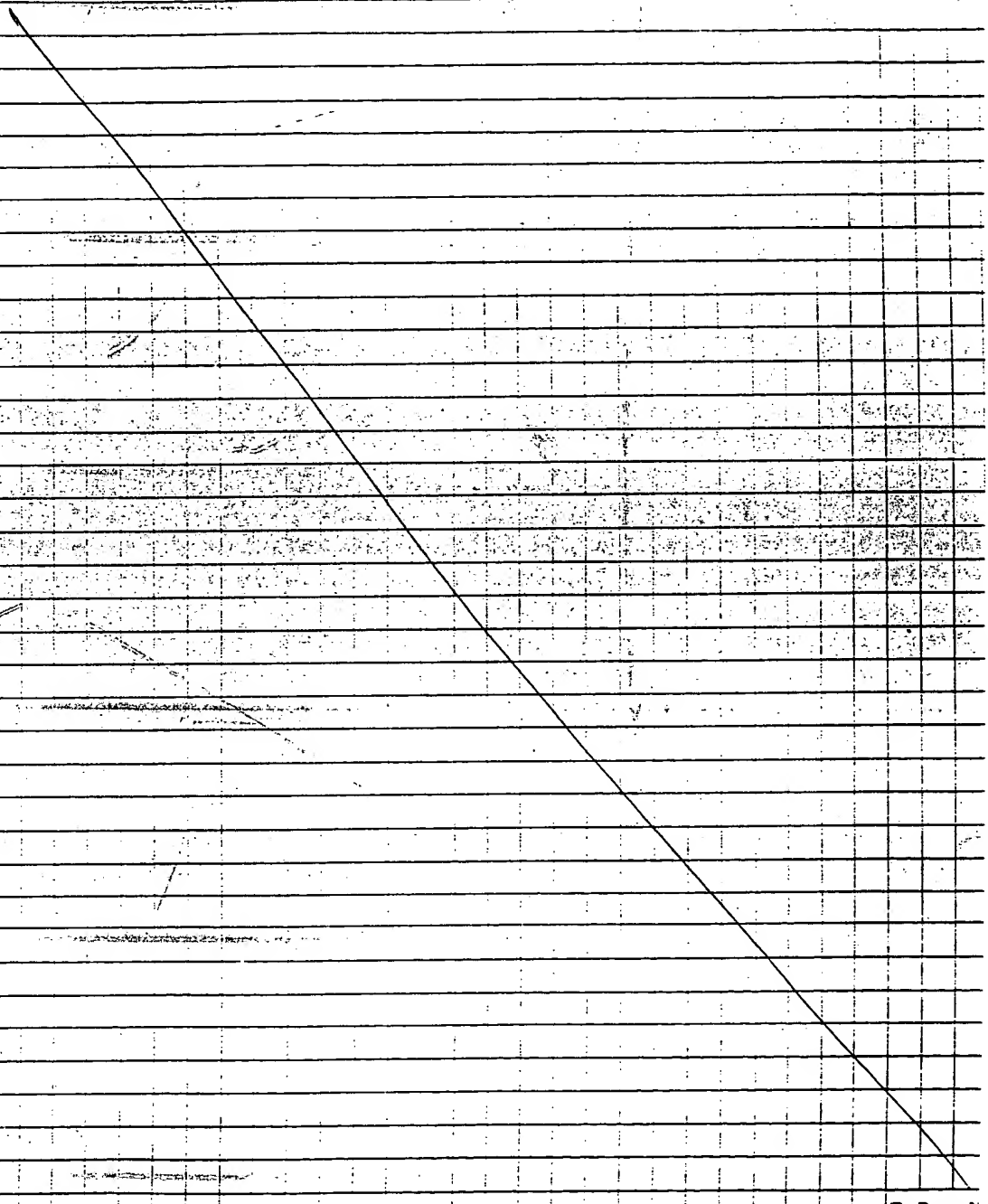

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			4/6/92

Project No. 78

Book No. 45 TITLE

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PROTOCOL FOR ELISA-HU IgG QUANTITATION

A) BUFFER SOLUTIONS

1) Coating Buffer: Carbonate buffer, 0.1M, pH=9.6
0.159 gm Sodium carbonate
0.093 gm Sodium bicarbonate
Adjust to pH 9.6, make up to 100 ml.

2) Washing Buffer: PBS, pH=7.4, with 0.1% Tween-20. (+Diluent)

3) Blocking Buffer: PBS, pH=7.4, with 3% non-fat dry milk.

B) PROCEDURE

1) Add antigen: Affinity purified Goat anti-human IgG (Fc fragment) (gamma chain specific) (Cappel cat. no. ~~[illegible]~~, lot no. 34760) at 1 ug/well in 50 ul coating buffer. Incubate at room temperature (RT), in a humid chamber for 2 hours. (#06010121 Rx# 55071)

2) Flick solution of the wells.

3) Add 0.2 ml blocking buffer for 30 minutes.

4) Add 0.05 ml of Hu IgG standard solutions: Human IgG (whole molecule, chromatographically purified, Cappel cat. no. 6001-0050, lot no. 34428).
Add 0.05 ml/well of the sample to be tested. Incubate for 1 hour, in a humid chamber at RT.

5) Wash 4X with washing buffer.

6) Add 0.05 ml of secondary antibody: Goat anti-human IgG peroxidase labeled (Cappel cat. no. ~~[illegible]~~, lot. no. 33083-5) diluted 1:1000 in PBS-.1% Tween-20. (# 86010081 Rx# 55261)
— RT 1hr.

7) Wash 4X with washing buffer.

8) Add 50 ul/well ABTS peroxidase substrate (Kirkegaard & Perry, cat. no. 50-64-02, lot. no. MH01). Incubate for 5 minutes, read at 405 nm.

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TITLE 13087 ELISA -9-10-91

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Human IgG Quantitation #1

10 September 1991

Follow procedures, P100

~~① Coat wells @ 1ug/well x 100 wells = 100ug/5ml DSP~~

① Coat plate c̄ Goat α Human IgG-Fc (from G. Bonsai)
- ⓐ Columns 1-4: 1ug/well -- 32ug/1.6ml
- ⓑ Columns 5-8: .5ug/well -- 16ug/1.6ml
- ⓒ Columns 9-12: .1ug/well -- 3.2ug/1.6ml

} Carbonate buffe[r]

-- Incubate, RT, 2 Hrs: 10^{15} - 12^{15}

② empty wells, add 200ul blocking soln; RT - 30'

③ Dilute Stds: 1mg/ml (1ug/ul) stock -- dilute 1:10 = 100ng/λ

320ng/λ: 3.2ul 100ng/λ → 1ml

-- serially dilute 1:2 ~~(350λ → 350λ)~~ (500λ → 500λ) to:

160 ng/ml
80 ng/ml
40 ng/ml
20 ng/ml
10 ng/ml
5 ng/ml

} in PBS, pH 7.4 / .1% Tween

④ Dilute Samples, 1:2 (200λ + 200λ) and 1:10 (40λ + 360λ)

⑤ Load wells

	1	2	3	4
A	C →		5566 916 1:2	
B	5 →		5566 916 1:10	
C	10 →		556461 916 1:2	
D	20 →		556461 916 1:10	
E	40 →		5536ML 916 1:2	
F	80 →		5536ML 916 1:10	
G	160 →		915 #6 1:2	
H	320 →		915 #6 1:10	

} repeat for 1ug/well, .5ug/well and .1ug/well of capture antibody

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Results

calculate standard curve using 0, 5, 10, 20 ng/ml standards
ETI calculator

1. punch in standard concentration (Y-value)
2. Hit X⇔Y key
3. punch in O.D. value (Y-value)
4. Hit Σ key
   -- repeat for all values to be used in standard curve
5. punch in sample OD * value
6. Hit X' key -- get value

Slope = .0098
correlation coefficient = .99
} using 0 → 20 ng/ml

Sample	1ng/well Value	.5ng/well	.1ng/well
SJ66 9/6 1:2	*~~37.4ng/ml~~ 74.8ng/ml	72.6ng/ml	66.6ng/ml
SJ66 9/6 1:10	66.1ng/ml *66.0ng/ml	--No results--	67.0ng/ml
SJ60/61 9/6 1:2	*30.6ng/ml 33.8ng/ml	30.4ng/ml	43.2ng/ml
SJ60/61 9/6 1:10	33.5ng/ml *39.0ng/ml	32.0ng/ml	28.0ng/ml
SJ36NL 9/6 1:2	0	0	0
SJ36NL 9/6 1:10	0	0	0
SJ60/61 8/15 10%DS 1:2	--off scale--	--off scale--	--off scale--
SJ60/61 8/15 10%DS 1:10	*367ng/ml	--off scale--	397ng/ml
Slope	*0.01157	0.0116	0.0125
correlation coefficient	*0.9965	0.9953	0.9726

*: 1ug/ml recalculated using 0 → 40 ng/ml
Standards in standard curve

SJ66 9/6 $\bar{X}$ = 69.4 ng/ml
SJ61/60 9/6 $\bar{X}$ = 33.8 ng/ml
SJ61/60 8/15 $\bar{X}$ = 382.0 ng/ml

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Project No. 98

TITLE 1308F Purification COS 8/9/91 Xfection Book No. 95

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1308F Purification: Protein G
COS 8/9/91 Transfection. NS Supes

2 October 1991
Thaw COS/1308F supes at ± 4°C

3 October 1991
Prepare Amicon MAC-Protein G capsule (item # 11039, 25mM d...

① Wash ē 20ml PBS, ~ 1ml/min.
② wash with 0.15M Glycine pH2.3, 15ml, ~1ml/min.
③ wash with 15ml PBS; elute pH 7.5 at end.

Filtered 1308F 10% NuSerum COS1 supe, 8.9.91 transfection, through .2μ Cellulose Acetate filter.

Load filter disk
4°C (cold room), using Rainin Rabbit peristaltic pump at 991
loaded 80ml sample @ 370.6ng/ml (#NB95-173);
80ml loaded in 60min (≈ 1.3ml/min)
Total sample = 29.6μg

Wash disk with 20ml PBS, 1.3ml/min; save PBS wash.

Eluted disk with 0.15M Glycine, pH2.3, 12ml. Collect 6 · 2ml aliquots into 15ml tubes containing ~ 1ml 2M Tris Base, pH unadjusted; eluted using syringe.

Read A_{280} of fractions:

1 - .275
2 - .033
3 - .020
4 - .015
5 - .015
6 - .015

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Project No.. 98
Book No. 2 TITLE 1308F Purificat... : Protein G

n Page No.

Dialyze samples #1 → #4, BRL 3/4" diameter MWCo 12-14kd prepared dialysis tubing, 3l PBS pH 7.4, 4°C.
4:15 PM → 6:15 PM

Change buffer; dialyze o/N.

October 1991

Remove samples to tubes -- ~5ml each.

Read A_{280}/1260:

Sample	1280	1260
1	.394	.436
2	.034	.039
3	.020	.025
4	.026	.029

Run 4-20% PAGE (reducing); 50λ sample + 10λ 4x loading buffer
-- 90°C 10'
-- load 50ul on Gel

Lane	Sample
1	Protein A Pre load
2	Protein A Flow thru
3	Protein A PBS wash
4	Protein A fraction 1
5	Rainbow Marker
6	Protein G PreLoad
7	Protein G flow thru
8	Protein G PBS Wash
9	Protein G fraction 1
10	Protein G fraction 2

Gel on LNB95-106.

Stain 1.5 hr, destain o/N, several changes.

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TITLE 1308F Purification: Protein G

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5 October 1991

filter fraction #1 through .2μ filter into 15ml tubes to 4°C

7 October 1991

IgG quantitation (by ELISA) LNB95-178:

Sample:	772 ng/ml
flow Thru:	603 ng/ml
PBS Wash:	0
Fraction 1:	0

Note: color development was poor in this assay. Also, applied sample 2 was the same as the applied sample for the Protein A column, which gave a value of only 240ng/ml (LNB95-108).

8 October 1991

Concentrate fraction 1 using Centricon 30 concentrator

1. load 2.3 ml
2. Spin J120, 3000 rpm, 30', 4°C
3. load another 2.0 ml, repeat step #2
4. final volume = ~~~400~~ DSP 350μl = 13 fold concentrate

ELISA

Protein G fraction 1: 0ng/ml

Protein G fraction 1 concentrate: 34ng/ml

The Ig seen on the gel is mostly Bovine Ig, which will adsorb protein G (FBS has ~920mg/ml Ig, = 920 ug/ml in 10% FBS med. so there is >1000 fold more bovine than 1308F Ig in the sample.

Only 13.5 ug (of a total of 61.8 ug) 1308F bound to the column. dialysis and concentration, only 11.9ng remained - a >1000 fold lo The possibility that the 1308F aggregated and was lost a filtering after dialysis can not be checked since there is pre-dialysis sample. Keep column flow thru, toss rest.

loss column flow thru
loss column wash
end fraction 1
DSP

Witnessed & Understood by me, | Date | Invented by | Date 10/8/91

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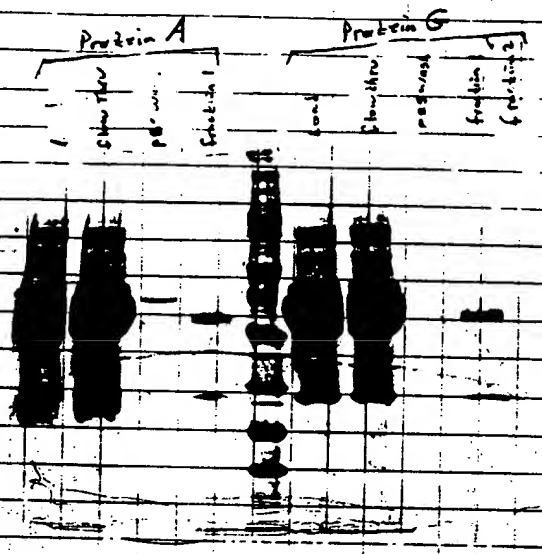
Project No. 98
Book No. 5 TITLE 1308F Purification: Protein G

m Page No.



LW895-104,107
10-4-91

To Rag No.

ess d & Understood by me, | Date | Invented by | Date

TITLE 1308F Purification & II Transfection; Protein A

Project No. 98
Book No. 95

From Page No.

1308F Purification: Protein A
COS 8.9.91 Transfection, NS Pooled Supes

4 October 1991

Prepared Amicon MAC Protein A Capsule (#11035)

① Wash with 25ml PBS, syringe
② Wash with 20ml .15M Glycine pH 2.3, syringe
③ Wash with 25ml PBS, syringe. Final eluate = pH 7.5

Loaded 80ml sample (LNB 95-103) onto Capsule by peristaltic pump, ~1.3ml/min, in cold room.
-- Total sample = 29.6 mg

Wash disk with 20ml PBS, save wash; A280 of last ml = .03

Elute with 12ml 0.15M Glycine pH 2.3; collect 2ml fract in tubes containing 1ml 2M Tris Base, pH unadjusted.

A280 of samples: 1- .191
2- .022
3- .013
4- .014
5- .016
6- .014

Dialyze fractions #1,2 against PBS at 4°C (3l):
① 9:40 → 12:15, change buffer
② 4°C o/n

Run Gel on Preload, Flow thru, PBS wash, Fraction #1 (@
-- LNB 95-104 for Gel info. Gel LNB95-106.

5 October 1991

Remove samples to 15ml tubes; 4°C o/n

To Pag

Witnessed & Understood by me, | Date | Invented by | Date 10-10-91

7 October 1991

IgG quantitation (by ELISA) LNB95-178:

Sample: 240ng/ml
Flow Thru: 35ng/ml
PBS Wash: 0
Fraction 1: 443ng/ml

Note: color development was slow and low. The applied sample was the same as for the Protein G applied sample (LNB95-105) which had a value of 772ng/ml.

8 October 1991

Concentrate fraction 1 using Centricon 30 concentrator:
① apply 2.3ml to concentrator
② spin 30', 3000 RPM, 4°C JA20
③ apply 2ml more, repeat step #2
④ final volume = 420 ml = 10 fold concentration

ELISA:
Protein A fraction 1: 58.3ng/ml
Protein A fraction 1 concentrated: 415.7ng/ml

Total sample bound to protein A = 16.4ug ([Sample - flowthru] x volume)
Total sample recovered = 2ug (443ng/ml x 4.5ml) = 12% recovery

The concentrated sample was ~~10~~ 7 fold more concentrated than the dialyzed sample, even though the volume was reduced 10 fold, indicating that some sample was lost to the concentrator. The dialyzed sample was 7 fold ~~more~~ less concentrated than the initial column fraction indicating that some of the sample was lost in dialysis -- adsorbed to the membrane or leakage.

Save concentrated fraction 1.

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Project No. 98
Book No. 5 TITLE 130RF 8.9.91 Transfection: Protein A #2

m Page No.

130RF 8.9.91 COS Transfection
Protein A Purification #2.

October 1991

PBS wash protein A disk, .15M pH 2.3 glycine wash, PBS wash.

Load Protein A (LUB 95-107) and Protein G (LUB 95-103) column.
Flow-Thru samples onto column -- 6ml/ml
---- Pass through column 3 times.

Wash column with 25ml PBS

Elute column with 12ml .15M Glycine pH 2.3 -- collect 3-2ml fractions into 1ml 2M Tris base.

Read A280 and A260 of fractions:

	A280	A260
1	.209	.171
2	.042	.050
3	.040	.049

-- Toss fractions 2 and 3.

Dialyze fraction 1 against PBS -- 2l @ 5:30 PM.
8:30 PM -- change buffer; 4°C o/n.

3 October 1991

Concentrate fraction 1 using Amicon centricon 30 concentrator
① load 2.3ml into concentrator
② Spin 3000RPM/30'/4°C J120
③ load additional 2ml, repeat step 2
④ final volume = 420ml = 10 fold concentration

ELISA

Protein A fraction 1 : 176ng/ml
Protein A fraction 1 concentrated : 425ng/ml

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tnessed & Understo d by me, | Date | Invented by | Date 10/9/91

TITLE 1308F 8·9·91 Transfection: Protein A #2

Project No. 198
Book No. 95

From Page No.

Again, the sample was concentrated 10 fold, but the 1308F u
concentrated only 2.4 fold -- must be adhering to the conc

Save Protein A2 fraction 1 concentrate.

To Page

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| | |

TITLE 1308F Purification: α Human IgG Agarose

Project No. 98
Book No. 95

10-7-91

From Page No.

10 October 1991

ELISA:

1308F NS Pool	-	219 ng/ml : 5ml aliquot
1308F NS Pool filtered	-	268 ng/ml : 5ml aliquot
Column Flow Thru	-	1 ng/ml
PBS Column Wash	-	1 ng/ml — Toss
PBS Column Wash #2	-	1 ng/ml
Fraction #1	-	60 ng/ml : 6ml
Fraction #2	-	0 ng/ml
Fraction #3	-	0 ng/ml — Toss
1M Glycine Strip	-	0 ng/ml

All the 1308F bound to the column (268 ng/ml on, 1 ng/ml flow Th
none eluted in the wash, and very little eluted in the
Elution strip or in the strip strip -- or all is being los
the dialysis step.

To Page

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TITLE 1308F Purification: Human IgG-Agarose (Tony's) Project No. 98 Book No. 95 10-18-91

From Page No.

1308F Purification
(Human IgG-Agarose Column (Tony)

18 October 1991

Will purify 1308F from 8.9.91 COS Transfection, NS Pool.

Pour ~1ml bed volume Anti-Human IgG-Agarose (from T. Ruiz) column, with PBS until UV trace is linear.

Load 200ml conditioned medium sample onto column by gravity flow (~2ml/min flow).

PBS wash until UV trace is linear.

Elute with 0.5M NH_4OAc pH3.0 (from Tony Ruiz); collect 2 fractions (~2.5ml each) and neutralize with .5ml 1M Tris

Remove 100ul aliquot from each sample -- store at 4°C.

Dialyze fraction #2 (~2.2ml) and ½ of fraction #1 (~2ml) aga 2l PBS o/n.

other ½ of fraction #1: buffer exchange on centricon microconcentrator:

1. apply 2ml sample to centricon 30
2. Spin 25min., 3000RPM, JA20, 4°C; volume = ~.5m
3. add 1.5ml PBS, pipette up and down to wash
4. spin 25min, 3000RPM, JA20, 4°C; volume = ~.8ml
5. add 1.5ml PBS, wash.
6. Spin 30min, 3000RPM, JA20, 4°C. Final volume = ~.
7. remove concentrated sample to new tube, 4°C. concentrator membrane with .3ml PBS, save at

19 October 1991

Change dialysis buffer.

21 October 1991

Remove dialysates to new tubes. filter sterilize #1 dialysates, and #1 concentrated with .2um low protein binding f

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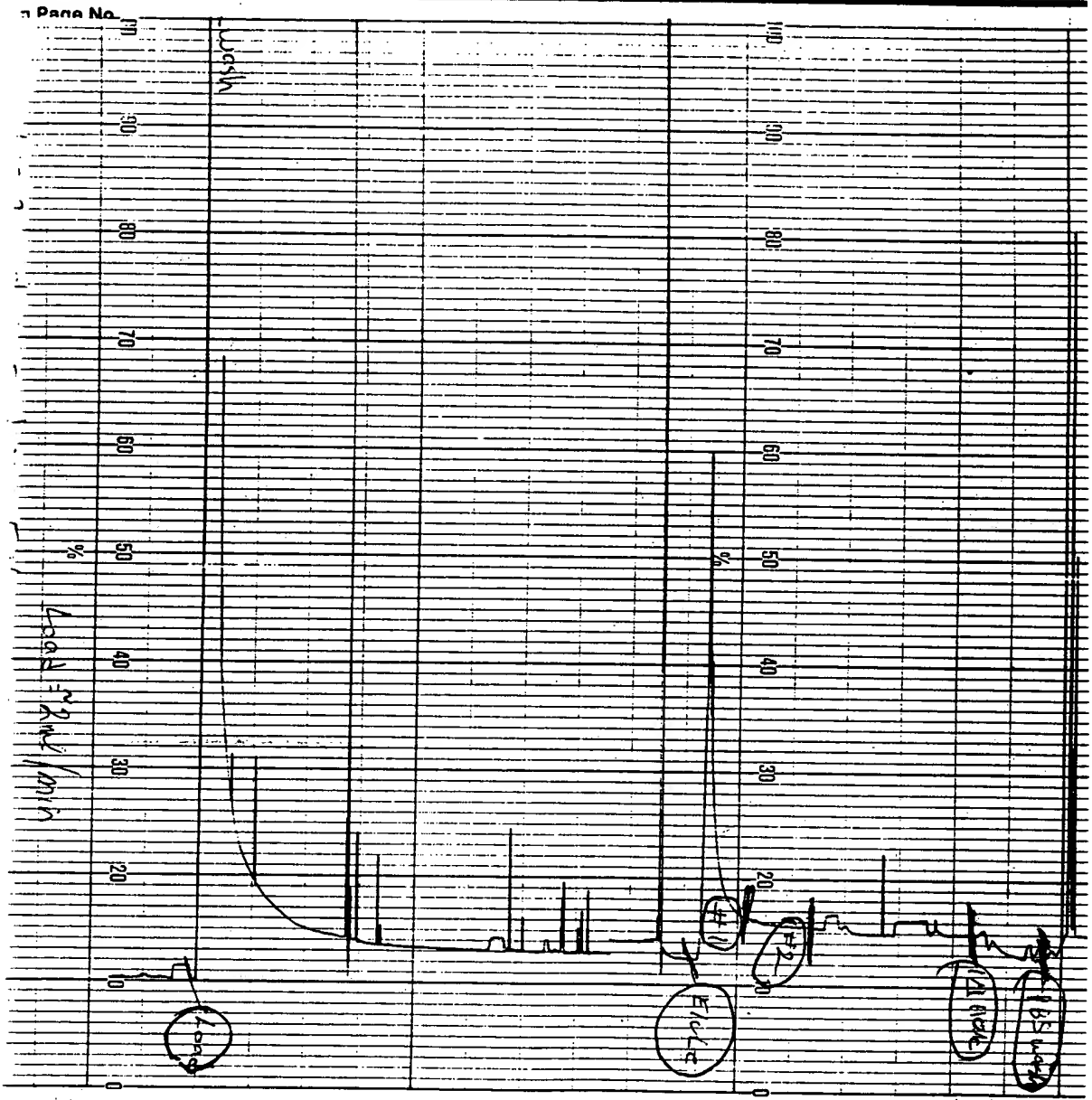
Project No. 98

Book No. 5 TITLE 1308F Purification of Human IgG-Agarose Co



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TITLE 1308F Purification: α" man IgG-Agarose (Tony's). Project No. 470 Book No. 95

From Page No.

22 October 1991

ELISA: (10/18, 10/21, 10/22):

Sample	Volume	[1308F]	Total 1308F	
Conditioned Medium	200 ml	202 ng/ml	40.4 µg	
Flow Thru	200 ml	14.7 ng/ml	2.9 µg	
Fraction #1	4.2 ml	3.7 µg/ml	15.5 µg	
Fraction #2	2 ml			
#1 dialyzed	2.4 ml	2.8 µg/ml	6.7 µg	
#1 dialyzed and filtered	2.4 ml	3.2 µg/ml	7.7 µg	*
#2 dialyzed	2.4 ml	.48 µg/ml	1.2 µg	
#2 dial. and filtered	2.4 ml	.82 µg/ml	2.0 µg	*
#1 Centricon	.75 ml	8.1 µg/ml	6.1 µg	
#1 Centricon filtered	.75 ml	8.8 µg/ml	6.6 µg	*

*Total recovery = 16.
40% recov

Using Tony's column resin and reagents, I can purify 1308F but with a poor recovery.

23 October 1991

Run 2 gels on purified material: 12.5% PAGE.

LO Customer

Lane
1 - 500 ng Murine 1308F
2 - 50 ng Murine 1308F
3 - Rainbow Markers
4 - 100 ng Purified 1308F
5 - 50 ng Purified 1308F
6 - Blank
7 - 10 ng Human IgG1, k
8 - 25 ng "
9 - 50 ng "
10 - 100 ng "

LO Company

Lane
1 - 500 ng Murine 1308F
2 - 50 ng " "
3 - Rainbow Markers
4 - 100 ng Purified 1308F
5 - 50 ng " "
6 - 10 ng Human IgG1, k
7 - 25 ng " "
8 - 50 ng " "
9 - 100 ng " "
10 - Blank

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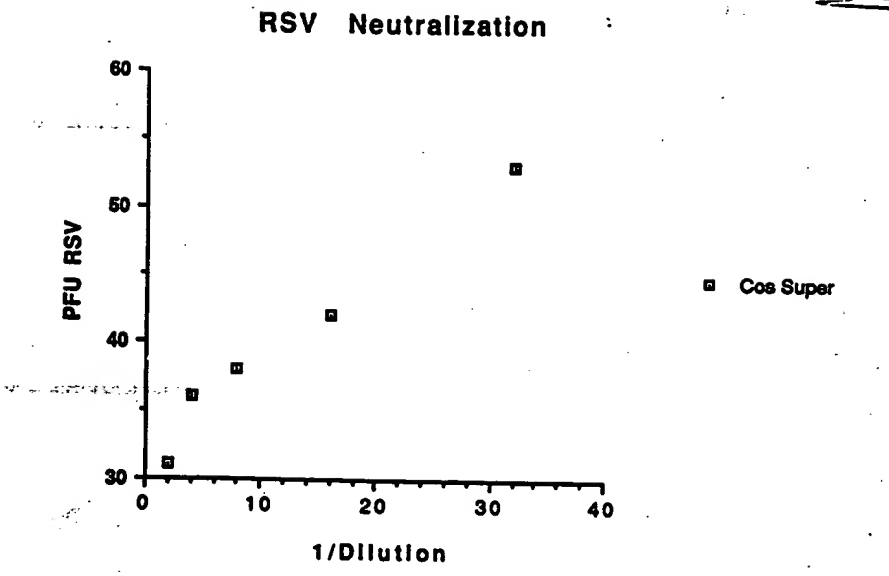
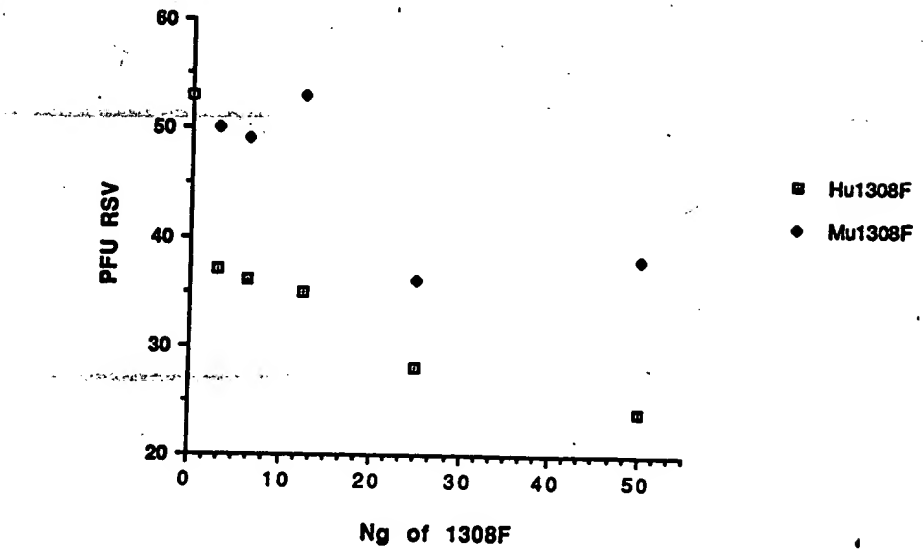
10/28/91


RSV Neutralization
PFU RSV
60
50
40
30
0
10
20
30
40
1/Dilution
Cos Super


PFU RSV
60
50
40
30
20
0
10
20
30
40
50
Ng of 1308F
Hu1308F
Mu1308F

1308F Purification
αHuman IgG-Agarose Comparison

22 October 1991

Pool 9/13/91 Cos transfection FBS supes (Blue):

SJ60/61	SJ66
9/13 → 9/16	9/13 → 9/16
9/16 → 9/20	9/16 → 9/20
9/13 → 9/27	9/13 → 9/27

-- add ½ of 8.9.91 10% FBS pool to each of the above po
(total volume = ~250ml each).

Wash 2 ~1ml bed volume columns (αHuman IgG-Agarose, one my lot other Tony's lot) with PBS.

Load columns simultaneously using peristaltic pump, ~3ml/min.
① equal volume through each column.
② pass flow thru over columns individually a second time.

Wash columns ≥ 20ml PBS

Elute columns with Tony's .5M NH_4OAc, pH 3
-- neutralize fractions to pH 7.0 with 2M Tris Base.

Dialyze samples (2 fractions each) against PBS O/N.

23 October 1991

Change dialysis buffer -- 2l @ 7:30 AM.
3:35 PM - remove samples to 15ml tubes; 4°C.

DSP Column, fraction #1 - 5.5ml
" fraction #2 - 6.5ml
Tony's Column, fraction #1 - 4.5ml
" fraction #2 - 2.3ml
} remove 0.2ml each to Sarstedt tubes; store @ -2

To Page N

Witnessed & Understood by m , Date Invented by Date

25 October 1991

ELISA:

	DSP column	Tony's Column
Column Flow Thru	63.5 ng/ml * (7.9 mg)	41.0 ng/ml * (5.1 mg)
Fraction #1	1.08 mg/ml (5.9 mg)	3.09 mg/ml (13.9 mg)
Fraction #1, dialyzed	1.19 mg/ml * (6.5 mg)	3.18 mg/ml * (14.3 mg)
Fraction #2	3.54 mg/ml (23 mg)	0 (0)
Fraction #2, dialyzed	2.91 mg/ml * (18.9 mg)	609 ng/ml * (1.4 mg)
	*Total: 33.3 mg	*Total: 20.8 mg

running (loading) the column at such a high flow rate decreased the efficiency of binding of the 1308F

8 October 1991

Combine DSP fractions 1 and 2 with Tony fractions 1 and 2:
~40 mg / 18 ml = 2.2 mg/ml

Save 3ml in 15ml tube, pass remainder over 0.3ml bed volume Protein A-Agarose column.

1. Pass flow Thru over column a second time
2. Wash column ε 4.5 ml PBS, save wash (15 bed vol
3. Elute column with 0.1 M Glycine pH 2.3
   - collect .25ml fractions into .25 ml 2M Tris Base
   - Dialyze against PBS in Microdialyzers, 350 vol, 2.5 hr

9 October 1991

ELISA

Sample	[1308F]	Volume	Total 1308F	%
Load 2.2 mg/ml ~~60 mg total~~		15 ml	33.3 mg	100%
Flow Thru	60.5 ng/ml	15 ml	0.91 mg	2.7%
PBS Wash	12 ng/ml	4 ml	0.048 mg	0.1%
fraction 1	20.1 mg/ml	0.45 ml	9.05 mg	27.2%
fraction 2	0.98 mg/ml	0.35 ml	.34 mg	1%
fraction 3	0		0	—
fraction 4	0		0	—
fraction 5	0		0	—
fraction 6	0		0	—
			0	—

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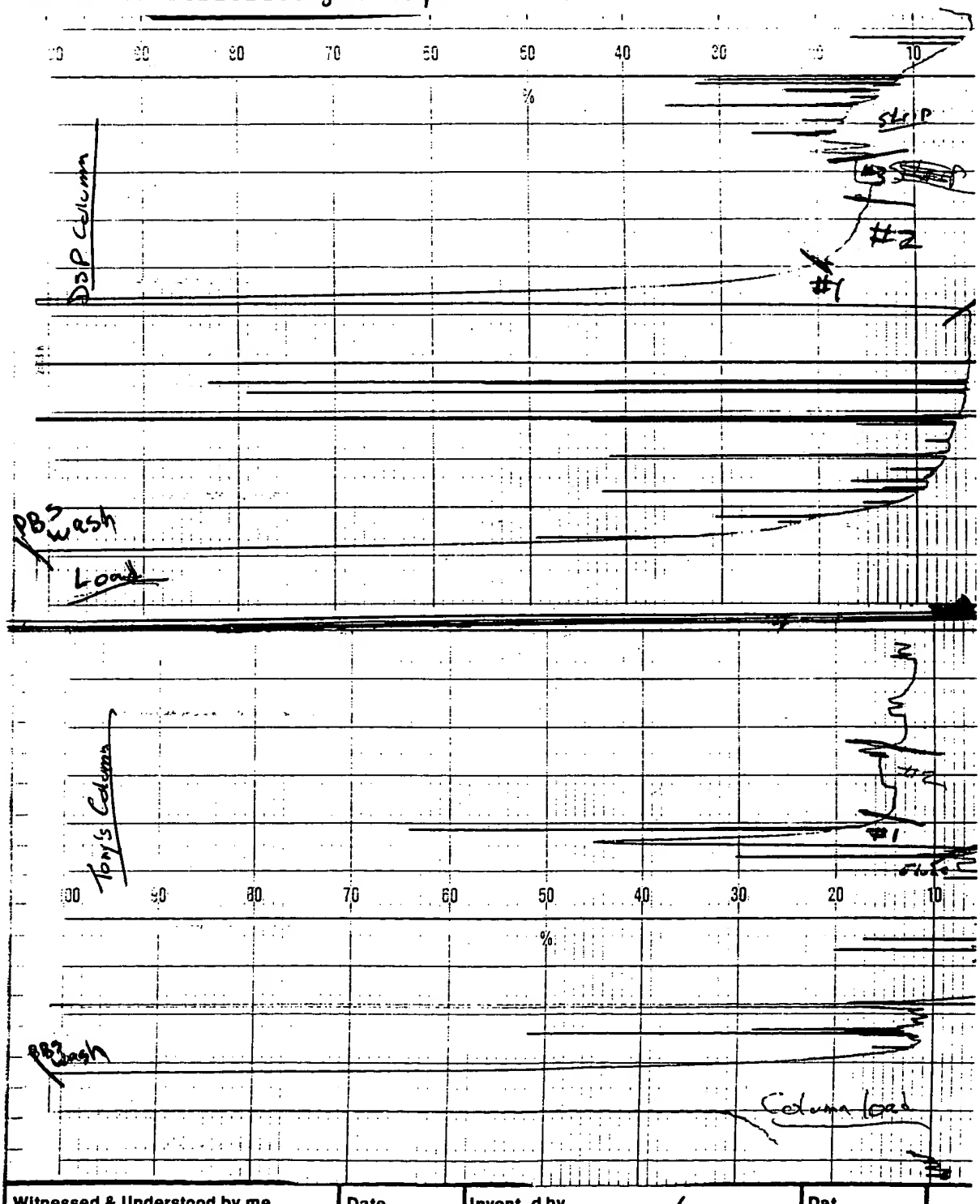
Prc t No. 98

TITLE 1308F Purification: αHuman Agarose Comparison

Book No. 95



Witnessed & Understood by me,	Date	Invent d by	Dat

of the 33ng of 1308F sample loaded onto the column, only ~ 10.3 ng (31%) can be accounted for. This loss is too great!

10 October 1991

Run 2 - 12% SDS/PAGE:

#1 - Coomasie Stain

Lane	Sample
3	Rainbow Standards
4	500ng Murine 1308F: 6λ 100ng/λ + 34λ 1x dye; load 33 ul
5	Blank
6	500ng Hu1308F Protein A fraction #1: 30λ 20.1mg/λ + 10λ 4x dye;
7	Blank
8	500ng Hu IgG1,K: 6λ 100ng/λ + 34λ 1x dye; load 33 ul.

#2 - Western Blot

Lane	Sample
1	Rainbow Standards
2	Hu1308F, 100ng -- α Human column (Tony) fraction #1 dialyzed; 63λ + 20λ 4x
3	Blank
4	H1308F, 100ng - α Human Protein A fraction #1: 10λ + 50λ 1x dye, load 30
5	Blank
6	200ng Hu-IgG1,K
7	100ng "
8	50ng "
9	25ng "
10	Blank "

Stain one gel, blot #2 to NC. Block blot o/N in 5% Milk/PB

November 1991

Wash blot, probe ~ GαH Ig-HRP. Wash. ECL Detect.

T Page No.__

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		[signature]	11/19/91

TITLE BC BF Purification: α Human / Pictrin A

Pr ct No. 98

Book No. 95

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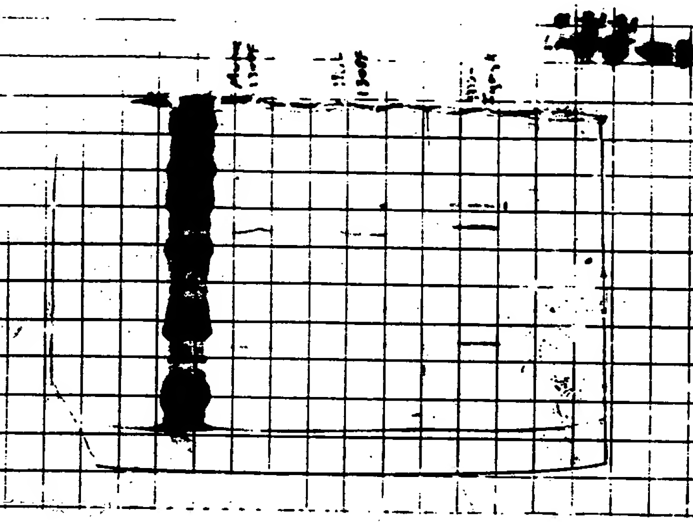

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TLE 1308F: SJ66/NS Pool Hu Purification

Project No. 98
Book No. 95

om Page No.

1308F: SJ66 Nu Serum Pool (COS)
αHuman Agarose Purification

6 November 1991

Pool all COS-Nu Serum-SJ66 supes remaining:

Teal 9/23 → 9/27	120 ml	
Teal 9/27 → 9/30	110 ml	
Teal 9/30 → 10/4	115 ml	(144 ng/ml)
Teal 10/4 → 10/8	115 ml	(172 ng/ml)

also combine with above COS-FBS-SJ66 supes:

Blue 9/20 → 9/23	80 ml	(149 ng/ml)
Teal 9/20 → 9/23	40 ml	
	580 ml Total	

Load ~1.5 ml bed volume αHuman IgG-Agarose column ← Pooled supe at 3.5 ml/minute

Wash Column ← PBS -- collect first 5 ml wash, continue to wa until UV baseline is stable (~40 ml total).

Elute column with 0.5 M NH_4Oac pH 3.0: 450 ml H_2O
14.5 ml Glacial Acetic Acid
NH_4OH to pH 3.0
-- bring up to 500 ml

-- collect ~3 ml fractions, neutralize to pH 7.5 with 2 M Tris base

Strip column with 1 M Acetic Acid -- collect eluate; elute ← mo 0.5 M NH_4Oac, collect ← 1 M strip. Adjust pH ← 2 M Tris Base.

Dialyze 3 fractions and column strip against 3 l PBS o/N

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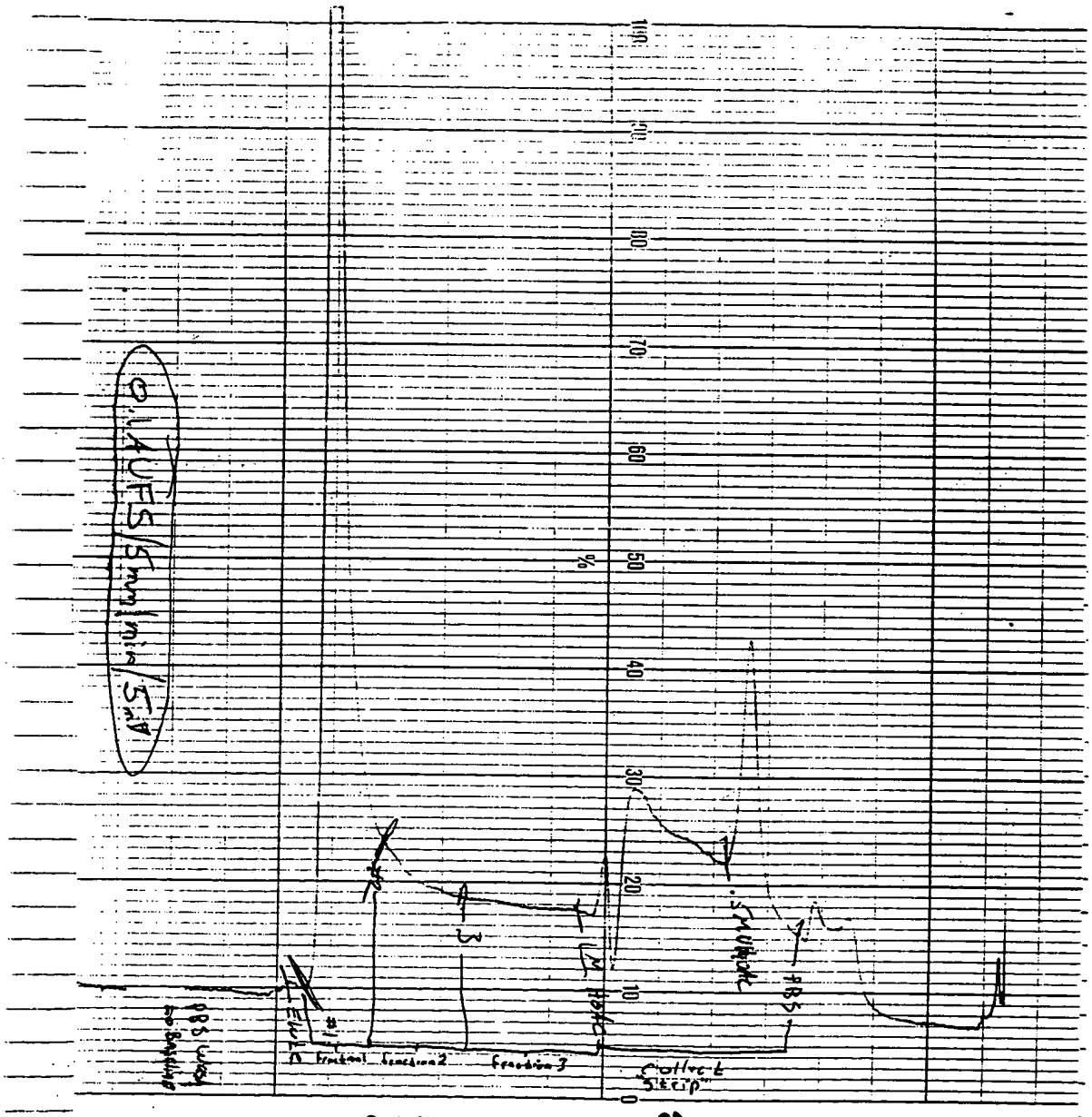
Witnessed & Understo d by me, | Date | Invent d by | Date 11/6/91

Project No. 98

Book No. 5 TITLE 13088: SJ66 Co. Pool of Human Purification

n Page No



Code No. 19-7288-01 Pharmacia

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TITLE 308F: SJ66COSPOOL 4 Human Purification

Project No. 98

Book No. 95

From Page No.

7 November 1991

Change dialysis buffer, dialyze ~~3 hrs~~ D50 O/N.

8 November 1991

.2 μm filter samples, to 4°C:

Fraction 1: 4.2 ml

Fraction 2: ~~4.6~~ 5 ml

Fraction 3: ~~6.7~~ ml

Column Strip: 11.6 ml

ELISA

Witnessed & Understood by me, | Date | Invented by | Date

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111

TITLE 1308F Purification SJ66 Fucia

Project No. 98
Book No. 95

From Page No.

1308F Purification
from SJ66 Fucia 10^{-7}M MTX lines 1, 2, 3

Want to purify a large quantity of hu1308F from ~~CEM~~ DSP Ch cells to use for ① K_D determination ② in vitro virus neutralization ③ in vivo virus neutralization in cotton rats.

ACE-00
DSP 2

10 January 1992

Count flasks of SJ66 Fucia 10^{-7}M MTX #1, #2, and #3:

#1 - 1.02×10^6 cells/ml × 140ml = 1.43×10^8 cells

#2 - 1.31×10^6 cells/ml × 140ml = 1.83×10^8 cells

#3 - 8.9×10^5 cells/ml × 150ml = 1.34×10^8 cells

--Note: these cells are clumped and the lar
clumps are not represented in these cell co
∴ these cell counts are underestimates of t
total viable cell #!!

SJ66 Fucia #1: seed entire volume into 500ml spinner wit
an additional 500ml R-/10%dFBS/L-Glu/10^{-7}M MTX; Volume
∴ have ≥ 2.23×10^5 cells/ml
P13-3.

SJ66 Fucia #2: seed entire volume into 1l spinner with an
additional 1l medium; Volume = 1140ml ∴ have ≥ 1.61
= P13-3

SJ66 Fucia #3: seed entire volume into 500ml spinner with an
500ml medium; Volume = 650ml ∴ have ≥ 2.06×10^5 cel
= P13-3.

--Cells seeded at 5:00 PM.

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Witnessed & Understood by me,	Date	Invented by	Date
			1/13/92

Project No. 98
Book No. 5 TITLE 1308F Purification: SJ66 Fucia

m Page No.

3 January 1992

Cell counts, 7^{30} AM (= ~62 hrs) and samples for ELISA:

		[IgG1,k]
SJ66 Fucia #1 :	5.2×10^5 cells/ml, 68% viable	8.1 ug/m
SJ66 Fucia #2 :	6.7×10^5 cells/ml, 94% viable	9.1 ug/m
SJ66 Fucia #3 :	4.9×10^5 cells/ml, 96% viable	14.2 ug/m

4 January 1992

Cell Counts (9^{00} AM) and samples for ELISA = ~88 hrs

		[IgG1,k]
SJ66 Fucia #1	1.1×10^5/ml, 16% viable	11.4 ug/ml
SJ66 Fucia #2	3.9×10^5/ml, 53% viable	11.2 ug/ml
SJ66 Fucia #3	2.5×10^5/ml, 61% viable	16.6 ug/ml

3^{00} PM (94 hrs): Spin cells 1200 RPM, 3', Beckman J6-B. .22 μ (ct) filter culture medium, store at 4°C o/

15 January 1992

- Pour ~8 ml bed volume Goat α Human IgG-Agarose (Sigma #A354: lot 059F-4883).
- Wash ē 2 bed volumes 4% Acetic Acid
- Wash ē 60 ml PBS, elute pH 7.4 at end
- load SJ66 Fucia #3 1/10 → 1/14 (16.6 ug/ml, ×640 ml) using Blue/yellow pump tubing, pump set at 790 = 4 ml/min.
- Wash ē PBS until UV trace returned to baseline
- Elute ē .5M NH_4Ac pH 3.0, collect 4 ~6 ml fractions, neutralize to pH 7.5-8.0 with 2M Tris base
- Wash column ē PBS
- Strip column ē 4% Acetic Acid; collect + neutralize elute

T Pag N 1

tnessed & Understood by me, | Date | Invented by | Dat 1/16/

TITLE 1308F Purification: 566 Fucia #3

Project No. 98

Book No. 95

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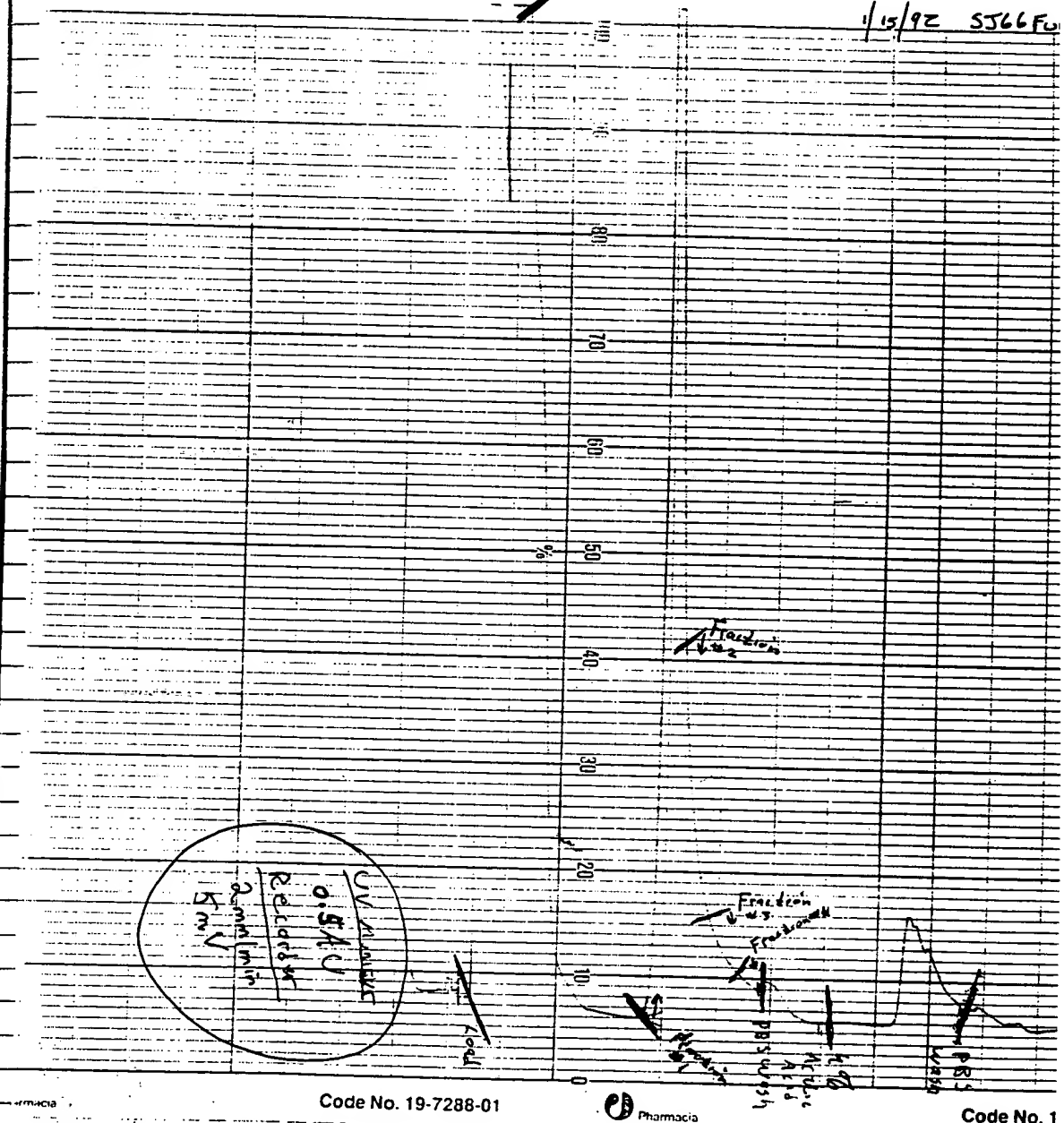

Code No. 19-7288-01 Pharmacia Code No. 1

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Project No. 98
Book No. - TITLE h1308F Purificat. : SJ66 Fxcia #3

m Page No.

- Dialyze 4 fractions and 4% HOAc Strip against 4l PBS, 4°C, 3½ h
- Change buffer, dialyze O/N.

January 1992

Assay samples

① Column Sample	13.77 mg/ml × 640 ml = 8.81 mg	
② Flow Thru #1	0.55 mg/ml × 400 ml = 0.22 mg	
③ Flow Thru #2	9.01 mg/ml × 240 ml = 2.16 mg	
④ fraction #1	370 ug/ml × 6.5 ml = 2.4 mg	
⑤ fraction #1 dialyzed	311 ug/ml × 7.7 ml = 2.39 mg	OD_{280} = .445 = 318 ug
⑥ fraction #2	89.7 ug/ml × 6.5 ml = 0.58 mg	
⑦ fraction #2 dialyzed	83.0 ug/ml × 8.4 ml = 0.7 mg	OD_{280} = .114 = 81 ug
⑧ fraction #3	32.4 ug/ml × 7 ml = 0.23 mg	
⑨ fraction #3 dialyzed	27.3 ug/ml × 9.5 ml = 0.26 mg	OD_{280} = .039 = 27.8
⑩ fraction #4	8.4 ug/ml × 4.5 ml = 0.038 mg	
⑪ fraction #4 dialyzed	7.0 ug/ml × 6.5 ml = 0.046 mg	OD_{280} = .022 = 15.7
⑫ 4% HOAc Strip	21.7 ug/ml × 7 ml = 0.152 mg	} Loss
⑬ 4% HOAc Strip dialyzed	21.3 ug/ml × 10.2 ml = 0.217 mg	}

Of the 8.81 mg total h1308F in the starting sample, only 5.99 mg (68%) can be accounted for in the flow thru and the fractions. Some may have been lost in the PBS wash, but 32% seems a bit high.

3.4 mg (39%) of the h1308F can be accounted for in the fractions collected. The trailing nature of the fractions may be due to too high of a flow rate during elution (4 ml/min). I will decrease the flow rate during the elution in the future.

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TITLE h1308F Purification SJ66 Fucia #3

Project No. 98
Book No. 95

From Page No.

(3) 4% of the h1308F came through in the 1st collected flow thru (the first loaded onto the column) and 65% came through in the 2nd collected flow (the last 240ml loaded). Apparently the column capacity was exceeded so late in the loading and all of the h1308F began to flow thru. I will more GαH Ig G-Agarose and pour a larger bed volume column in the future.

SJ66 Fucia #3 Flow Thru Purification

Since there is 2.38 mg (27%) of the starting h1308F in the flow thr will attempt to purify it.

-- Rinse the GαH Ig G-Agarose column ≈ PBS, strip ≈ 4% HOAc, and rinse with PBS.

-- Load the 1st + 2nd column Flow thru from 1/15/92 onto the column at 2.5ml (Blue-Yellow pump tube, setting 500). Start at 5:15 PM, stop at 9:20

-- Wash the column ≈ 60ml PBS, UV trace to baseline.

-- Elute slowly (setting 350) ≈ 0.5M NH4OAc pH 3.0, spike elution buf ≈ ~25% 4% HOAc after the second fraction (i.e. for fraction 3). Collect fractions.

-- Strip column ≈ 4% HOAc, wash ≈ PBS, store in PBS/Azide.

-- Dialyze 3 fractions (fraction 1 = ~~the front~~ DSP ~10ml, fraction 2 = ~4ml, fract ~9.5ml) against 2l PBS o/n 4°C.

17 January 1992

Change dialysis buffer, dialyze 3hrs, 4°C.

Remove samples to 15ml polypropylene tubes:

fraction 1 = 11.4ml
fraction 2 = 8.3ml
fraction 3 = 11.7ml

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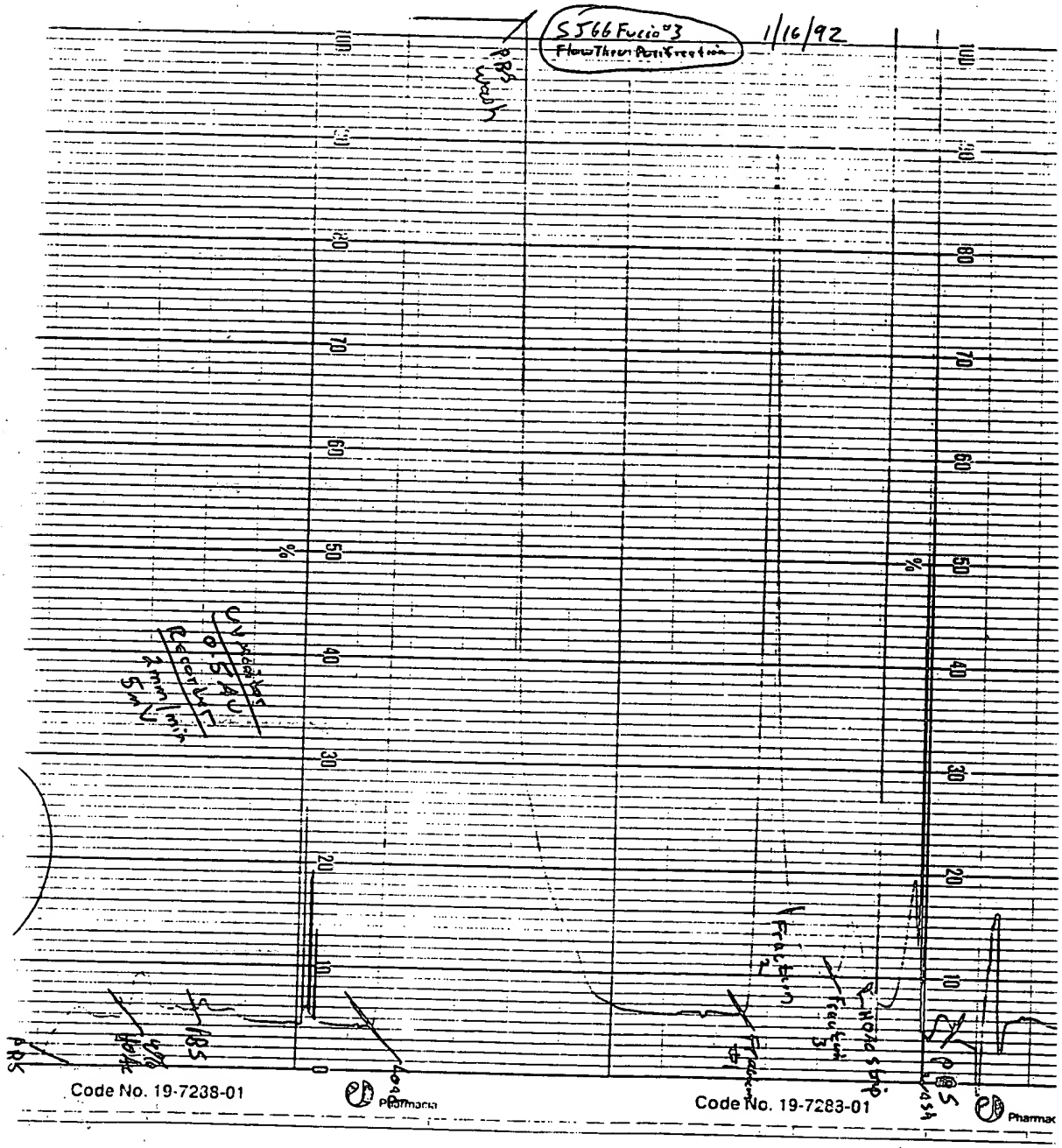
Project No. 98

Book No. 5 TITLE 1308F Purification. SJ66 Fucia #3



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TITLE 1308F Purification SJ66 Fucia #3 and #1 Project No. 98 Book No. 95

From Page No.

Assay:

① Column Sample: 4.01 μg/ml × 640 ml = 2.57 mg

② Flow Thru: 0 μg/ml

⑤ Fraction 1: 101.8 μg/ml × 11.4 ml = 1.16 mg
A280 = .202 = 1.44 μg/ml

④ Fraction 2: 10.8 μg/ml × 8.3 ml = 0.09 mg
A280 = .026 = 18.6 μg/ml

⑤ Fraction 3: 6.0 μg/ml × 11.7 ml = 0.07 mg

— dialyzed Samples

-- Slower elution rate might be giving a tighter elution, but since this c wasn't as heavily loaded as the 1/16 column it is not a fair comparison.

-- Only 51% recovery (1.32 mg) of the input sample was attained

-- Toss fraction #3, since its elution was aided by the addition of 4%

SJ66 Fucia #1 Purification

Will purify the H1308F from the SJ66 Fucia #1 conditioned medium (assayed at 11.4 μg/ml × 600 ml = 6.8 mg). Will elute the column with 3M K. to see ① if it gives a better elution profile and ② if the product substantially different than the .5M NH4Ac pH3 eluted material.

-- Rinse GAHIgG-Agarose column (8 ml bed volume) with 60 ml PBS, wash c 4% HOAc, rinse c ~ 50 ml PBS -- pH 7.4 at end.

-- load Column at ~2.5 ml/min (blue-yellow tubing, 500).

-- Wash column c ~100 ml PBS, UV trace to baseline.

-- Elute slowly (setting 320) with 3M KSCN. Collect 3 fractio

-- Dialyze fractions 4 hrs against 2 l PBS.

-- Change buffer, dialyze o/n.

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Project No. 98

Book No. 5 TITLE 1308F Purification: SJ66 Fucia #1

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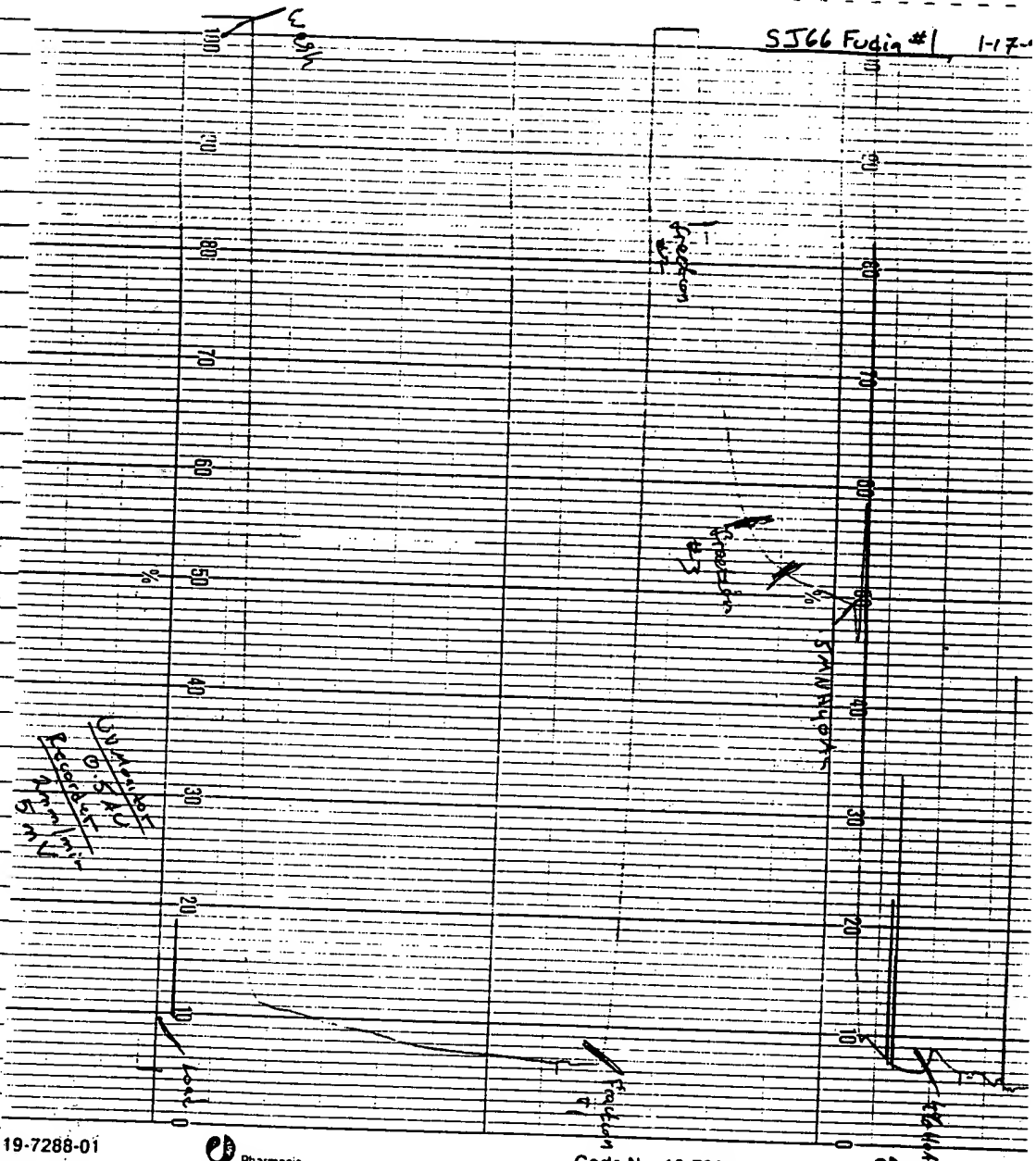
SJ66 Fudia #1 1-17-



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Invented by Date 11/21

TITLE 1308F Purification: SJ66 Fucia #1 and #3

Project No. 98
Book No. 95

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20 January 1992

Assay

① Sample: 8.92 µg/ml × 600ml = 5.35 mg

② Flow Thru #1: 2.15 µg/ml × 410ml = 0.88 mg

③ Flow Thru #2: 15.23 µg/ml × 190ml = 2.89 mg

④ Fraction #1: 7.47 µg/ml × 9.5ml = 7.1 mg

⑤ Fraction #2: 119 µg/ml × 9.0ml = 1.07 mg — Dialyzed Samples

⑥ Fraction #3: 26.75 µg/ml × 6.7ml = 0.18 mg

-- Even at a slow flow rate the capacity of the column is exceeded, so it is apparent capacity of the column that is a problem and not the flow rate of loadin

-- 8.35 mg were eluted from the column and 3.77 mg flowed through the col but there were only 5.35 mg in the starting sample. Apparently some of t material "lost" from the SJ66 Fucia #3 runs was never eluted from the col with .5M NH4OAc pH3 or 4% acetic acid, but it did elute with 3M KSCN. If is the case, then these samples may not be useful since some of the protein them have been subjected to several rounds of acid washing.

21 January 1992

Concentrate samples: SJ66 Fucia #3 fraction #1 (LNB95-136)

Test Centriprep concentrators (Amicon #4306)

① Make Human IgG Soln: 0.6ml @ 17.8 mg/ml → 42.5ml = 25 µg/ml

OD_{280} = .321 = 229 µg/ml

② load 11ml 229 µg/ml into centriprep 30 (2.519 mg)

③ Spin 10 min, 2400 RPM, Beckman GPR tabletop centrifuge

④ decant filtrate, spin an additional 9 min as above.

⑤ Remove retentate: 2.2ml, OD_{280} = 1.411

= 1.01 mg/ml × 2.2ml = 2.22 mg = 88% recovery

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Project No. 78
Book No. 45 TITLE ~~SJ~~ 1308F Purification: SJ66 Fucia #3

n Page No.

Wash Centriprep 30 ~ PBS:
(1) load 5ml PBS
(2) Spin 10', 2400 RPM, decant filtrate + retentate.

SJ66 Fucia #3 GαHIgG Agarose Fraction #1 (LWB95-136) Concentration:
(1) load 7.5ml onto prewashed Centriprep 30, Spin 10', 2400 RPM
(2) decant filtrate (4.5ml), repeat spin, 4'.
(3) Remove Retentate: Final volume = 1ml A_{280} (1:40) = .076
= 2.17mg/ml * 1ml = 91% recovery

A_{280} retentate (undiluted) = .006

SJ66 Fucia #3 GαHIgG Agarose Fraction #2 and #3 Concentration (LWB95-136)
(1) load fraction #2 (8.2ml * 8 mg/ml) + fraction #3 (9.9ml * 27.8 mg/ml) into same centriprep 30 that fraction #1 was concentrated on.
(2) Spin 10', 2400 RPM, decant 6.5ml filtrate
(3) Spin 10', 2400 RPM, decant filtrate (5.5ml) } 14.5ml filtrate total
(4) Spin 5', 2400 RPM, decant 2.5ml filtrate } A_{280} filtrate = .003, toss
(5) Add SJ66 Fucia #3 Flow Thru Purification fraction #1 (LWB95-139) to the retentate cup (11.2ml * 144 mg/ml). Spin 10', 2400 RPM, decant.
(6) Spin 10', 2400 RPM, decant.
(7) Spin 5', 2400 RPM, decant. OD_{280} filtrate = .004
Retentate = 1.8ml OD_{280} = 1.85 = 1.32 mg/ml
= 2.38mg total = 94% recovery.

→ Pool SJ66 Fucia #3 fraction #1 concentrate with SJ66 Fucia #3 fraction 2+3 / SJ66 Fucia #3 flow thru fraction #1 concentrate:
(2.17mg/ml * 1ml) + (1.32mg/ml * 1.8ml) = 3.49mg / 2.8ml
= 1.25mg/ml
-- .22 micron filter (Amicon #4454) Ab prep, aliquot in 5 - 0.5ml aliquots and one 0.3ml aliquot.

-- OD_{280} = 2.12 = 1.51 mg/ml

Labeled: h1308F Top:
95-142
1.21.92

Tube1: Jim Tomura 1.22.92
Tube2: Charles Riggin 1.23.9
Tube3:
Tube4:
Tube5:
Tube 6: DS Purse.

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tnessed & Understood by me, Date Invented by Date

TITLE 1308F Purification SJ66 Fucia #2 Project No. 98 Book No. 95

From Page No.

SJ66 Fucia #2 Purification

I will purify the h1308F from ½ of the SJ66Fucia #2 ½-½4 conditioned medium, a higher capacity column and KSCN elution.

-- Increase bed volume of the GxH IgG-Agarose column to 10ml with fr resin (~~BRL~~ ISP (Sigma 13543 lot 059F-4883). Rinse column with PBS.

-- Strip column with 20ml ~~X~~ DEP 3/1 KSCN, rinse with 50ml PBS.

-- Load column, RT, 4ml/min (blue-yellow tube, 790); collect flow thru in 2 aliquots, #1 ~280ml and #2 ~250ml. Collect the very last 2ml thru in seperate tube labeled "Terminal Flow Thru"

-- Wash column ~ ~70ml PBS to stable baseline; Note: the UV baseline 3/M KSCN is ~ 4.5 x that of PBS (PBS baseline @ 6%; KSCN @ 50%). Base line was adjusted for KSCN, recorder set to 2mV for PBS ba to be seen.

-- Elute ~ 3/M KSCN, collect 4 fractions of 3-6ml.

-- dialyze fractions against 2l PBS, 4°c, o/N.

22 January 1992

Change dialysis buffer, continue dialysis for 4 hrs.

Remove samples to 15ml polypropylene tubes; read OD of samples and of dialy buffer, blanked against PBS:

① Dialysis buffer - 0 - .005

fraction 1 : OD_{280} = .122 = 87 µg/ml × 2ml = 174 µg
fraction 2 : OD_{280} = .922 = 659 µg/ml × 5.2ml = 3.42 mg
fraction 3 : OD_{280} = .313 = 224 µg/ml × 4.0ml = 894 µg
fraction 4 : OD_{280} = .121 = 86 µg/ml × 5.4ml = 468 µg

Total = 4.96 m

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Book No. 0 TITLE 1308F Purification: SJ66 Fucia #2

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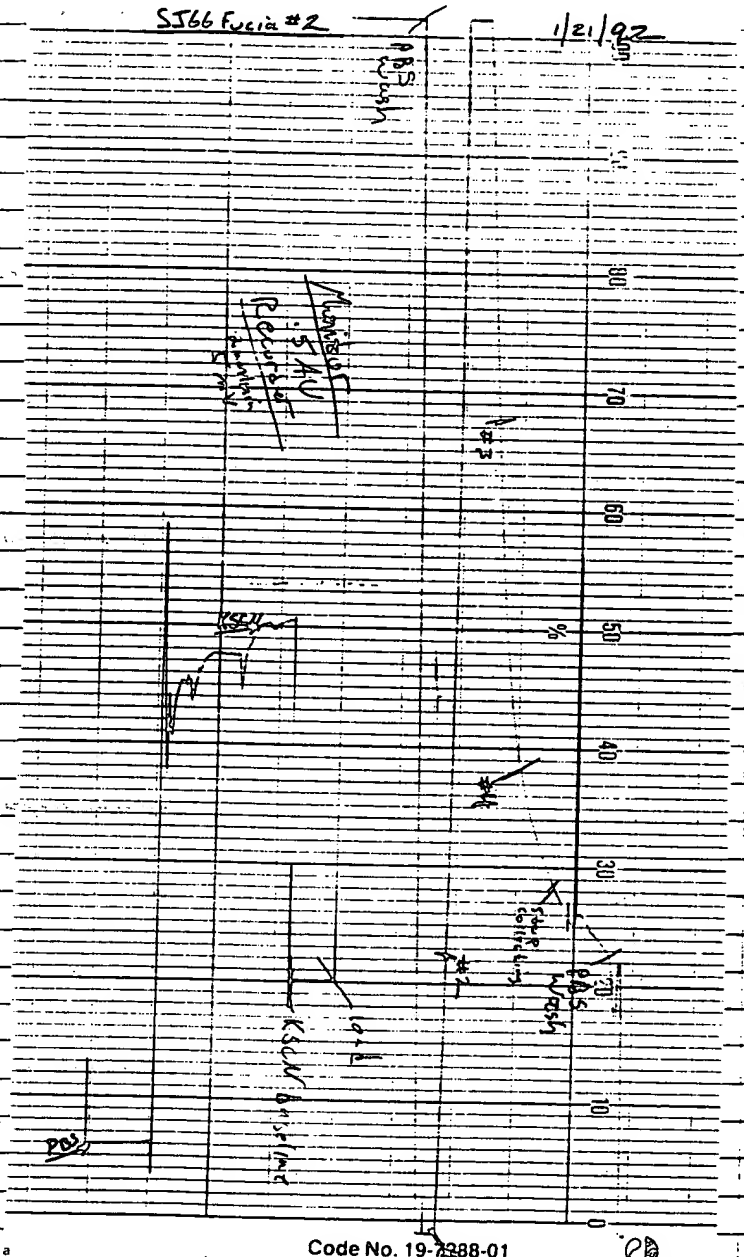
SJ66 Fucia #2 1/21/92



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TITLE 1308F Purification . . JG6 Fucia #2

Project No. 98

Book No. 95

From Page No.

Pool SJ66 Fucia #2 GG4 IgG & Agarose fractions #1, 2, 3, 4: 4.96mg / 16.6ml

① Spin 10', 2400 RPM, 4°C, Beckman GPGR in Centriprep 30

② decant 3.4ml filtrate: OD_{280} = .004, toss.

③ Spin 10', 2400, 4°C. Decant 3ml filtrate: OD_{280} = .002, toss.

④ Spin 15', 2400, 4°C. Decant 3.1ml filtrate: OD_{280} = .000, toss

⑤ Spin 15', 2400, 4°C. Decant 2.7ml filtrate: OD_{280} = .000, toss.

⑥ Spin 5', 2400, 4°C. Remove 3.7ml retentate, OD_{280} = 1.909 = 1.36mg/ml

1.36mg/ml × 3.7ml = 5.03mg

Filter sterilize (Gelman #4454, .2um), aliquot in 6 - 0.5ml and 1 - 0.3ml aliquots.

Read OD_{280} = ~~3.6~~ 1.80 = 1.29mg/ml

Label: h1308F
95-145
1-22-92

Top: [redacted]

Tube 1: Jim Tamura 1-22-
Tube 2: Charles Riggin
Tube 3: Geetha Bansal
Tube 4: Geetha Bansal
Tube 5: Geetha Bansal
Tube 6:
Tube 7: DSP use

23 January 1992

Humanized 1308F Purification Preparations 1/92

Prep: h1308F 95-142

Purified: [illegible] - [illegible] Goat & Human IgG-Agarose
0.5M Alanine pH 8.0 dialyzed
2M Di. deionized
dialyzed against PBS
Centriprep 30 concentrated
0.2um filtered

Concentration: 1.51mg/ml

Aliquots: 5 x 0.5ml, 1 x 0.3ml

Distribution: Tube 1 to GJT 1-22-92
Tube 2 to CHR 1-23-92

Prep: h1308F 95-145

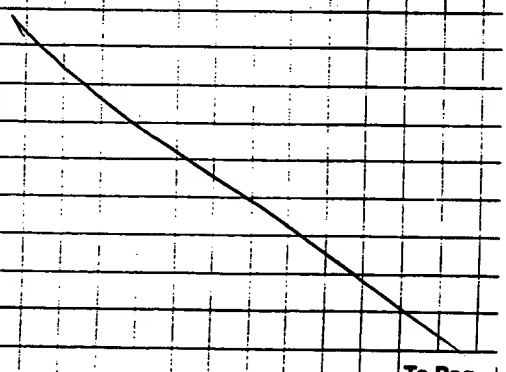
Purified: [illegible] - [illegible] Goat & Human IgG-Agarose
2M KSCN eluted
dialyzed against PBS
Centriprep 30 concentrated
0.2um filtered

Concentration: 1.29mg/ml

Aliquots: 6 x 0.5ml, 1 x 0.3ml

Distribution: Tube 1 to JT 1-22-92
Tube 2 to CHR 1-23-92

Summary of purificat
for Syd.



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Project No. 98

46 Book No. 95 TITLE 1308F Purific. Lots: SJ66 Fusion #2, #3

From Page No.

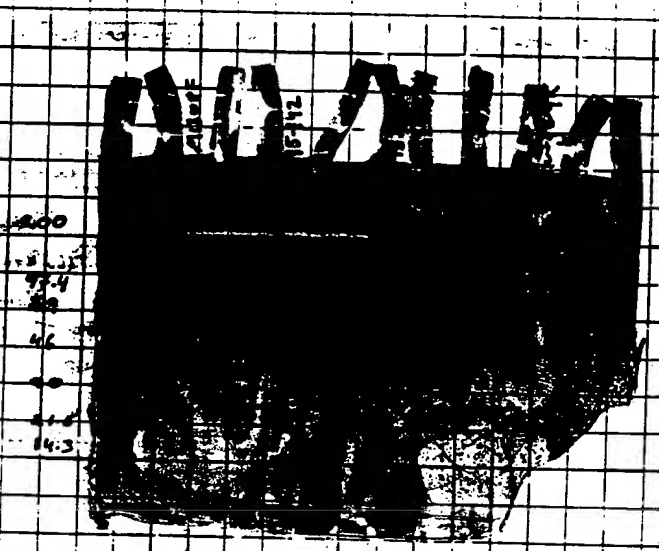
24 January 1992

load 1ug each of 95-142 and 95-145 on 4-15% Gradient SDS-PAGE, along w. 1ug Murine 1308F and 1ug HuIgG1,k standard. Run 1hr, 115 volts. Stain 1hr, destain o/n.

25 January 1992

Change destain, shake o/n.

27 January 1992



Even though by Lisa equ amounts of MW 4 and 1 were loaded (1ug), there seems to be less protein in the 95-145 lane. Also, 95-145 has a small (+)ok contaminant not seen in other lane.

Note: MSC did RIP against labeled, RSV infected, HEP2 cells on 1/24 both 95-142 and 95-145 immunoprecipitate RSV-F protein. 95-142 brought down more RSV-F than did 95-145 (equal amounts of MAb used) which can be explained by the discrepancy in concentrations seen in the above gel.

28 January 1992

Note: JT performed a competition ELISA, F protein down, using M1308F and 95-142 and 95-145, detecting with αMouse-HRP. Both 95-142 and 95-145 compete with M1308F for binding to RSV-F b/c with an apparent K_d ~200 fold lower than M1308F.

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TITLE 1308F Purification: SJ66 Fucia #2 + #3

Project No. 98

Book No. 95

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Assay Results (Assay done 1.13.92)

SJ66 Fucia #2 Purification

① Sample: 11.28 ug/ml

② Flow Thru #1: -0-

③ Flow Thru #2: 1.87 ug/ml — Toss These.

④ final 3ml of Flow Thru: 2.86 ug/ml

⑤ Purified, concentrated h1308F95-145: 1.01 mg/ml

-- Apparently the capacity of the column is low, since all of the Ab is abs
out of the first 280ml of sample, but as the loading continued
Ab was absorbed by the column.

-- The ELISA shows only 1.01 mg/ml H1308F in the final, concentrated sam
whereas by A_{280} there is 1.29 mg/ml. Part of this discrepancy may
due to the <10kd contaminant

-- h1308F95-142 is 1.89 mg/ml by ELISA but only 1.51 mg/ml by A_{280}
ELISA and A_{280} readings need to be repeated for both preps

14 February 1992

Confirmatory ELISA

H1308F 95-142: 3.1 mg/ml (@ 1:40,000 dilution)

H1308F 95-145: 1.60 mg/ml (@ 1:20,000 dilution) } 1.72 mg/ml
1.84 mg/ml (@ 1:40,000 dilution)

-- these results are quite different than earlier ELISA results and
A_{280} results. Will have to repeat these on another ELISA

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3 Book No.. 5 TITLE H1308F Purification: 95-142, 95-145

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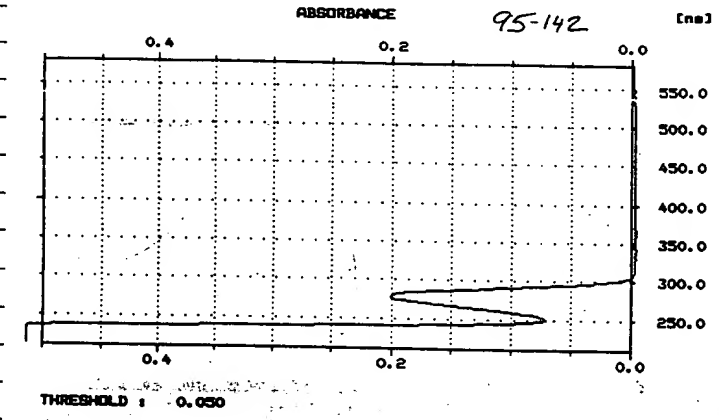
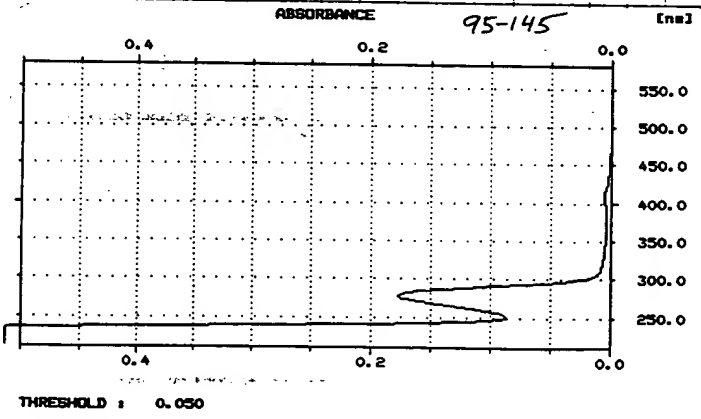
20 February 1992

Scan 1:10 dilutions of 95-142 and 95-145, 210nm → 380nm:





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Humanized 1308F Purification Preparations 1/92

ep: h1308F
95-142

fied: 1/15/92 - 1/21/92 Goat α Human IgG-Agarose
0.5M NH_4OAc pH 3.0 Eluted
2M Tris Neutralized
dialyzed against PBS
Centriprep 30 concentrated
.2μm filtered

entration: 1.51 mg/ml

uots: 5 × 0.5ml, 1 × 0.3ml

ribution: Tube 1 to JJT 1.22.92
Tube 2 to CHR 1.23.92

p: h1308F
95-145

fied: 1/21/92 - 1/22/92 Goat α Human IgG-Agarose
3M KSCN Eluted
dialyzed against PBS
Centriprep 30 concentrated
.2μm filtered

ntration: 1.29 mg/ml

uots: 6 × 0.5ml, 1 × 0.3ml

ribution: Tube 1 to JT 1-22-92
Tube 2 to CHR 1.23.92
Tube 3+4 to GB 2.20.92

TITLE H1308F Purification, 5-142, 95-145

ject No. 98
Book No. 95

From Page No.

dilute 95-145 DSP Add 4 µg of each 1:10 dilution to a tube (31.75 λ 95-142, 27.6 λ 95-142), bring to 40 µl ~~µl~~ PBS, add 10 µl 4X Protein sample buffer to each.

ELISA on 95-142 and 95-145:

		A_{280} (2.2-0.2)
95-142 :	2.70 mg/ml	1.45 mg/ml
95-145 :	1.27 mg/ml	1.28 mg/ml

Quantitation Results

95-142		95-145	
ELISA	A_{280}	ELISA	A_{280}
1.89 mg/ml	1.51 mg/ml	1.01 mg/ml	1.29 mg/ml
3.10 mg/ml	—	1.72 mg/ml	—
2.70 mg/ml	1.45 mg/ml	1.27 mg/ml	1.28 mg/ml
$\bar{x}$ = 2.56 mg/ml	$\bar{x}$ = 1.48 mg/ml	$\bar{x}$ = 1.33 mg/ml	$\bar{x}$ = 1.29 mg/ml

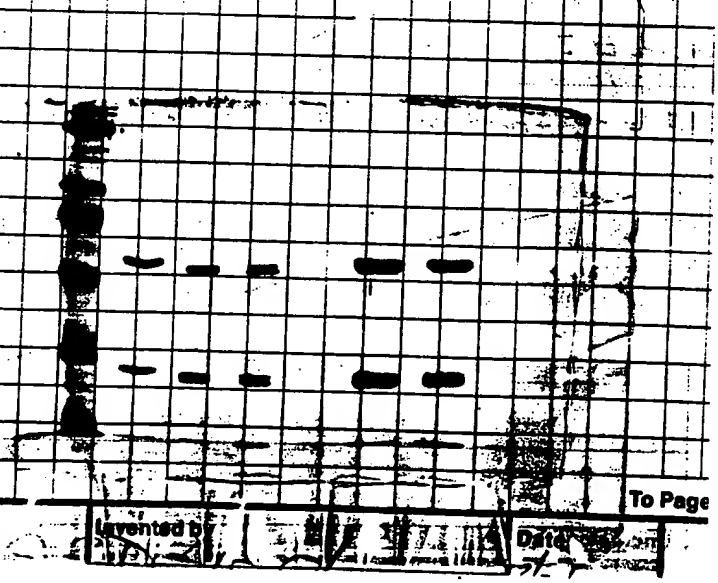
H1308F 95-1
= 1.3 mg

The ELISA and A_{280} for 95-142 are quite different, while they agree fairly well for 95-145.

21 February 1992

Want to look at 95-142 and 95-145 on protein gel to see if they are pure and intact still. Run 15% SDS-PAGE:

Lane	Sample
1	Rainbow Markers
2	1µg HIgG1,κ
3	1µg 95-142
4	1µg 95-145
5	empty
6	3µg 95-142
7	3µg 95-145
8	—
9	—
10	—



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Project No. 98
Book No. 5 TITLE CH1129 and H1308F-S71 Purification

n Page No.

Purification
CH1129 and H1308F-S71

March 1992

Thaw COS supes (LNB95-53-56) at 4°C O/N

H1308F-S71

2/11-2/14	37ml @ 2.78 μg/ml =	103 μg	326 μg total/115ml
2/14-2/17	36ml @ 3.90 μg/ml =	140 μg	
2/17-2/20	42ml @ 1.97 μg/ml =	83 μg	

CH1129

2/11-2/14	26ml @ 4.45 μg/ml =	116 μg	375 μg total/86ml
2/14-2/17	28ml @ 5.90 μg/ml =	165 μg	
2/17-2/20	32ml @ 2.93 μg/ml =	94 μg	

March 1992

- Pour 2 1ml bed volume GαH IgG-Agarose (Sigma) columns, wash with PBS, load samples by gravity flow (~3ml/min).
- Wash columns ⊂ PBS
- Elute ⊂ 3M KSCN (3M in H_2O, pH ~7.5 unadjusted).
- Collect 3 fractions of H1308F-S71, 4 fractions of CH1129.
- Dialyze fractions 1.5 hr against PBS in microdialyzer.

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ness d & Understood by me, | Date | Invented by D. S. Rose | Date 3/9/92

TITLE CH1129 and H1308, 571 Purification

Project No. 98

Book No. 95

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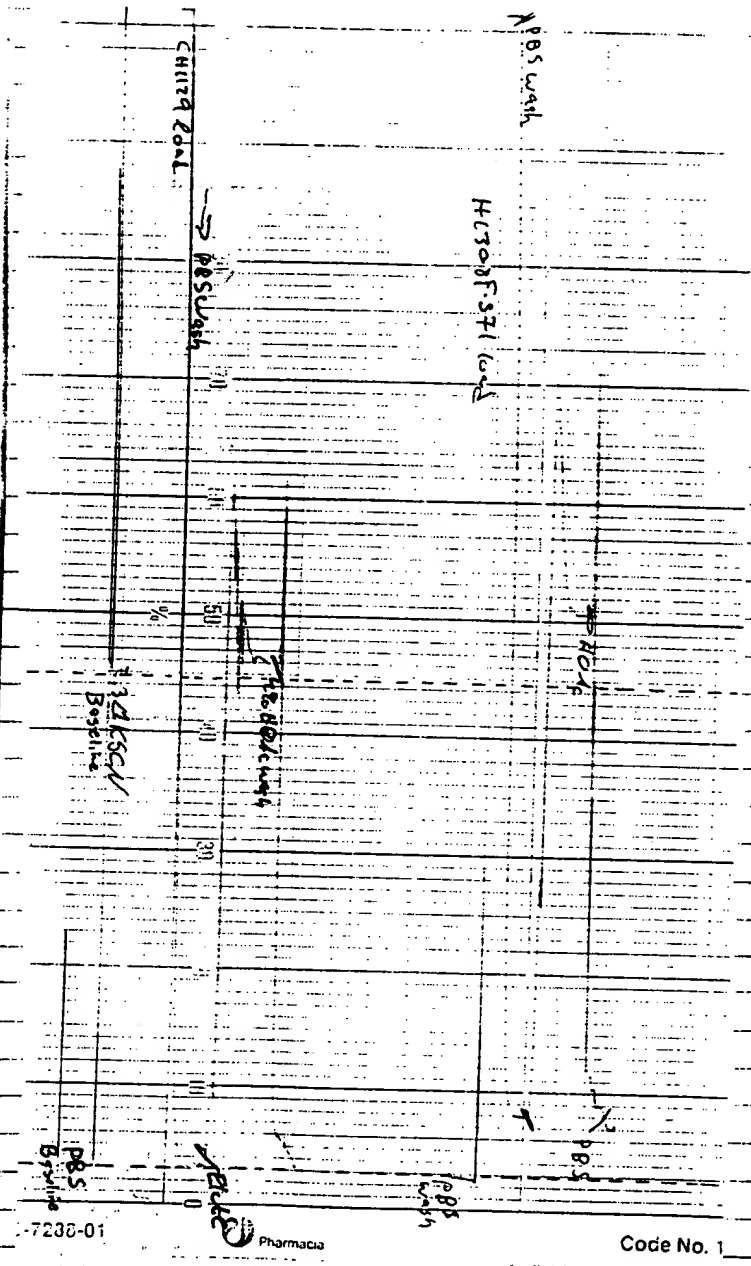

Scale - .5AUFS
Sp-1 - 2 mm/min

-7238-01 Pharmacia

Code No. 1

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Project No. 98

Book No. 5 TITLE CH1129 and H1.8F.571 Purification

Page No.

March 1992

ELISA

	CH1129	H1308F.571
Column Sample	4.8 mg/ml x 86 ml = 413 mg (100%)	3.3 mg/ml x 115 ml = 380 mg (100%)
Flow Thru	0.91 mg/ml x 86 ml = 78 mg (19%)	0.66 mg/ml x 115 ml = 80 mg (21%)
fraction 1	29.2 mg/ml x .7 ml = 20 mg (5%)	0.0 mg/ml x
fraction 2	24.1 mg/ml x .9 ml = 22 mg (5%)	23.4 mg/ml x 1.8 ml = 42 mg (11%)
fraction 3	17.8 mg/ml x 1.5 ml = 27 mg (7%)	13.7 mg/ml x 1.5 ml = 21 mg (6%)
fraction 4	9.4 mg/ml x 2 ml = 19 mg (5%)	N/A

CH1129: pool fractions 1, 2, 3, 4; dialyze against 2 l PBS 3 hr, change buffer, dialyze O/N

H1308F-571: pool fractions 2 + 3; dialyze against 2 l PBS 3 hr, change buffer, dialyze O/N

March 1992

Remove samples to tubes; to 4°C.

March 1992

ELISA (1:50, 1:100)

CH1129: 9.2 mg/ml x 6 ml = 55.2 mg

H1308F.571: 13.8 mg/ml x 3.5 ml = 48.3 mg

3 April 1992

Confirmatory ELISA: .2 µm filter CH1129: 9.77 µg/ml

T Page No.

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n Page No.__

M&b Western

April 1992

Transfer 0.5mg each M&b. to a clean microfuge tube:

694 (pST106/107 #2, 4.6.92
98-6 (MEU98-6h+b #2, 4.6.92
447 (447/13 4.6.92

H1129 (5x transfection, 5.29.92, LNB195-69) 2mg/ml
CH1129 (pST186, 3.27.92, LNB95-67) 2.37mg/ml
CH1129, purified (LNB95-150) 9.2 mg/ml -- 54µl + 200µl PBS

H1308F.971, purified (LNB95-150) 13.8 mg/ml -- 36µl + 200µl PBS
H1308F.68-71 (2.5x + cx pooled transfections; LNB118-70, 3.20.92)
H1308F, purified (LNB95-145, from 1:10 dilution 2.20.92), 13mg/ml -- 4µl + 200µl PBS

COS Mock supe - 1ml

April 1992

Add 50µl Pansorbin cells to each tube, rotate 4°C 1.5hr.

Spin cells, decant, resuspend in 0.5ml M&b. buffer
Spin, decant, resuspend in 200µl RIPA
Spin, decant, resuspend in 200µl RIPA, transfer to new tube
Spin, decant.
Resuspend in 100µl 1X Sample loading buffer
Boil 10'
Spin 5'
Load 25µl onto Daiichi 4-20% gradient SDS-PAGE:

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| | |

TITLE MAb Western

Project No. 83.98
Book No. 95

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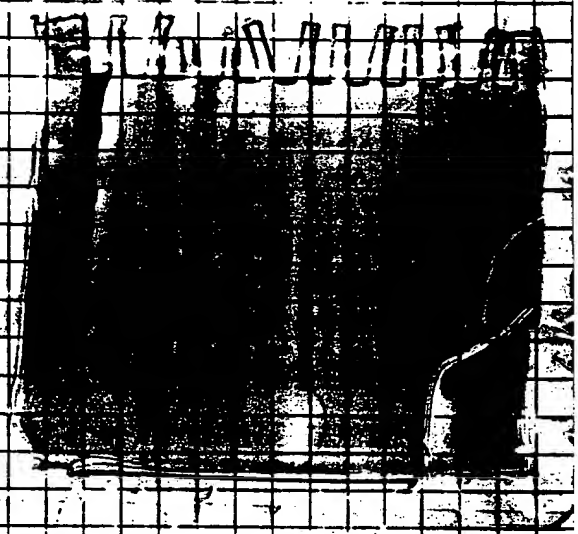
Lane	Sample
1	MW stds
2	Cos. Mock
3	447
4	694
5	98-6
6	H1129
7	CH1129
8	CH1129, purified
9	H1308F·66→71
10	H1308F·571 purified
11	H1308F purified, 95-145
12	empty



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TITLE 1308F Conditioned Medium: COS

Project No. 98
Book No. 95

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1308F/COS Transfection 8/9/91
-- Conditioned Medium

12 September 1991

Transfection Date 8/9/91

Plasmid SJ 60/SJ 61

Serum	Harvest Date	Time on Cells	Volume	Antibody Concentration	Total Antibody	
FBS	8/12/91	72 Hr	75ml	492ng/ul	36.7ug	
SF	8/14/91	48 Hr	15ml	64ng/ml	1ug	
NS	8/15/91	48 Hr	30ml	283ng/ml	8.5ug	103.1
FBS	8/15/91	48 Hr	30ml	322ng/ml	9.7ug	
NS	8/19/91	72 Hr	120ml	238ng/ml	28.6ug	
NS	8/21/91	72 Hr	120ml	155ng/ml	18.6ug	

-- Total Antibody = 103.1g.

Thaw Supes, combine FBS containing and combine NS containing supes:

"Stated" volumes by C. Schmidt
- FBS - 46.4ug/105ml = .44ug/ml
- NS - 55.7ug/270ml = .206ug/ml
- Mock (FBS - 77ml

Combine Supes, measuring 25ml pipet:

FBS
- 8/15/91 - 27ml
- 8/12/91 - 26ml + 39ml
- 92ml

remove .5ml aliquot

NS
- 8/15/91 - 26ml
- 8/19/91 - 112ml
- 8/21/91 - 98ml
- 236ml

remove .5ml aliquot

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Book No. 2-5 TITLE 1308F Conditioned Medium: COS

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13 September 1991

Filter 3.5ml of each (Mock, NS, FBS) supe through an Accrodisc 0.2μm Low-Protein-Binding syringe filter to Sterilize (product # 4192, Gelman Sciences).

-- Remove 0.5ml, give remaining 3ml to C. Riggins for virus neutralization assay.

ELISA on pooled supes, pre- and post-filtering

-- ABTS development very slow; read after ~ 12min of developing - no development in IgG, standard lanes, very little in IgG lanes. Develop another 10'. -- still poor development

-- Wash plate 1X = PBS-Tween, develop with TMBlue, stop with 4.5% H_2SO_4. Much higher development of the IgG lanes, still very poor development of the IgG, lanes

Generate standard curve using TMBlue data, standards 0 → 6.75
correlation coefficient = .998
Slope = 0.80

Mock = 0
Mock filtered = 0

1308F NS = 16.2 ng/ml
1308F NS filtered = 16.7 ng/ml

1308F FBS = 31.3 ng/ml
1308F FBS filtered = 31.6 ng/ml

These results are way lower than expected from previous assays (by G. Bansal / T. Ruiz).

An error must have been made in diluting the standards -- Hey it's Friday the 13th so what do you expect?!!

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TITLE 1308F Conditioned Medium: COS (repeat of 9/3) Project No. 98 Book No. 95

From Page No. 1308F/COS Transfection (8/9/91)

Conditioned Medium - Pre and Post Filtering

•16 September 1991

-- repeat of assay, 9/13/91.

Standards

-- 3 sets: ① IgG1 from T. Ruiz
② IgG (diluted to 100ng/ml) on 9/10/91 from A. Bonsal
③ IgG received from Cappel and diluted to 1mg/ml on 9/12/91.

Results

Standard Curves

IgG1	IgG (from 9/10/91)
0 - .066	0 - .074
1.56 - .075	1.56 - .085
3.125 - .090	3.125 - .110
6.25 - .125	6.25 - .141
12.5 - .187	12.5 - .258
25 - .301	25 - .461
50 - .547	50 - .784
100 - .968	100 - 1.298
Slope = .0091	Slope = .0125
Correlation Coefficient = .9994	Correlation Coefficient = .9955

-- new IgG standards are way off scale!! Looking back at the spec. the ~~Sample~~ stock solution is actually 17.8 mg/ml !!

	IgG1 Stds	IgG Stds	%dIgG1
1308F FBS Pool	878.4 ng/ml	586.9 ng/ml	50%
1308F FBS Pool - Filtered	871.8 ng/ml	582.1 ng/ml	50%
1308F NS Pool	419.9 ng/ml	267.9 ng/ml	57%
1308F NS Pool - filtered	370.6 ng/ml	232.1 ng/ml	60%
9/16 SJ66 supe #3 -	648.8 ng/ml	437.2 ng/ml	48%
9/16 SJ60/SJ61 supe #8 -	890.1 ng/ml	612.7 ng/ml	45%

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Book No. 5 TITLE 1308F/COS quantitation 9/13/91 XL

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9/13/91 SJ66 and SJ60/61 Transfection

September 1991

Coat plate: .5ug/well GaHIgG Fc (DSP stock) in Carbonate buffer
-- 4°C o/n

September 1991

Dilute Standards: IgG1, IgG (Immunology), IgG (DSP stock).

IgG1

Slope = .0131
Ccoef = .9972

1:20	x̄	1:40
0	0	0
0	0	0
644	636	627
822	841	860
615	605	594
536	595	603
569	550	530
316	846	875
723	718	707
830	813	796
877	346	314
786	861	936

IgG (Immunology)

slope = .0206
Ccoef. = .9945

	1:20	x̄	1:40
1	0	0	0
2	0	0	0
3	382	364	345
4	496	494	492
5	364	344	323
6	345	337	329
7	335	309	282
8	492	447	302
9	436	416	395
10	501	477	452
11	531	497	463
12	473	507	541

IgG (DSP)

Slope = .0185
Ccoef. = .9943

	1:20	x̄	1:40	%
1	0	0	0	
2	0	0	0	
3	418	394	370	8
4	544	539	534	9
5	397	372	346	8
6	377	365	352	8
7	365	353	300	8
8	540	542	544	9
9	477	452	426	9
10	550	519	488	9
11	583	542	501	9
12	518	553	588	9

There is an 8-9% difference between Immunology's IgG standards and my IgG standards; mine should be fine to use hereafter.

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TITLE 1308F/COS 9/13/.. xfection

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From Page No.	IgG1	IgG	%1
1 - Mock	0	0	—
2 - Mock	0	0	—
3 - SJ66	636	379	68%
4 - SJ66	841	517	63%
5 - SJ66	605	358	69%
6 - SJ66	595	351	70%
7 - SJ66	550	321	71%
8 - SJ60/61	846	520	63%
9 - SJ60/61	718	434	65%
10 - SJ60/61	813	498	63%
11 - SJ60/61	846	520	63%
12 - SJ60/61	861	530	62%

Average 1308F Production		
~~Stock~~	IgG1	IgG
SJ66	645 mg/ml	385 mg/ml
SJ60/61	817 mg/ml	500 mg/ml

During the first 72 hrs. of production after the transfection, it appears that the co-transfection (SJ yields about 28% more 1308F than the single transfec of SJ66.

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Project No .98
Book No. 15 TITLE 1308F/cos 9/91 xfection

om Page No.__ 9/13/91 xfection, 9/16-9/20 Supe Quantitation

20 September 1991

Dilute IgG1 and DSPs IgG standards.

Run assay -- standard curve calculation via softmax software.

Cos Samples, T150's, seeded 9/16/91; pooled:

Sample	IgG1	IgG	%A	NS/FBS	
				IgG1	IgG
SJ66 - FBS	359 ng/ml	173 ng/ml	75%	140%	137%
SJ66 - NS	501 ng/ml	237 ng/ml	114%		
SJ60/61 - FBS	283 ng/ml	141 ng/ml	101%	120%	118%
SJ60/61 - NS	340 ng/ml	167 ng/ml	104%		

1308F Production SJ66 : SJ60/61		
	IgG1	IgG
FBS	127%	123%
NS	147%	142%

* Note: P100's were scrape and seeded into T150's 9/16/91; cells had low viabi and were not very dense

(1) Cells seem to be producing more 1308F in 10% NS than in 10% F

(2) Later in the transfection (sec 8175) the SJ66 gives higher production than the cotransfected SJ60 and SJ61. This could due to unequal amounts of SJ60 and SJ61 in the cells.

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Witnessed & Understood by me, | Date | Invented by | Date 9/20/91

TITLE 1308F/COS 9/13/91 Transfection Pro No. 98
Book No. 95

From Page No.— 9/13/91 CosTransfection: ELISA
9/27 → 9/30 Sopus

30 September 1991
Dilute IgG1 and IgG standards (10ng/ml ~~stds~~ stocks from 9/20/91.

Samples: Mock NS Mock FBS
SJ66 NS SJ66 FBS
SJ60/61 NS SJ60/61 FBS

--2 Samples from T. Ruiz, 1:50 and 1:500.

Results
Standards are 10 fold higher than what is shown on cov. sheet --- mis-diluted first standard 1:10 (1ng/ml) instead of 1:100 (100ng/ml).

IgG-1 Standards 0-125ng/ml
Slope = .005
Correlation Coefficient = .997

IgG Standards 0-125ng/ml
Slope = .007
Correlation Coefficient = .991

--all Cos samples (1:10 and 1:20) are Zero!

Witnessed & Understood by m , Date Invented by Date

Project No. ___

Book No. ___ TITLE 1308F Quantita. :: Purification #1

Page No. 1308F 8.9.91 Purification: ELISA

October 1991

Dilute Tony Ruiz's IgG1 to 100ng/ml, serially dilute 1:2

Rec'd new Human IgG1,k (Sigma #I 3889, lot #80H8965).

-- Dilute in 1ml PBS (~1mg/ml)

-- dilute an aliquot 1:10 (100µl → 900µl PBS), read A_{280}/ A_{260}:

$A_{280} = .189$

$A_{260} = .133$

Protein concentration = $1.5(.189) - .75(.133) = 184 \mu g/ml$

-- Dilute to 100ng/ml, serially dilute 1:2

Extinction coefficient for immunoglobulins is 1.4

∴ 1L 1:10 dilution = $.189/1.4 = .135 mg/ml = 135 \mu g/ml$

Stock IgG = 1.35mg/ml

Aliquot 50ml/Tube @ 1.35mg/ml IgG1,K

-- Store @ -20°C

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Read & Understood by me, | Date | Invented by | Date 11/7/91